Comparative Morphology of Hematopoietic Neoplasms

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Comparative Morphology of Hematopoietic Neoplasms

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Comparative Aspects of Hematopoietic Neoplasms of Animals and Man—Summary
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PREFACE

The aim of this Monograph is to bring together current knowledge and concepts about the morphology of hematopoietic neoplasms developed in three different areas of research. As more and more has been learned about these neoplasms in domestic animals, in laboratory animals, and in man, different backgrounds have engendered different emphases and even quite different vocabularies. Synthesis of investigative results from the three fields is necessary and imminent. Morphologic pathology seemed an excellent place to begin the process. Different, incompatible—even conflicting—terminologies have concealed both analogies and provocative species differences. Besides trying to remedy this, the Monograph is designed to serve as a single convenient reference source for pathological information from the different areas. We hope that the reports as well as the conference that generated them also will serve as catalysts for more effective communication between the various pathologists studying veterinary, laboratory, and human leukemias and lymphomas.

JOHN W. BERG

INTRODUCTION

The search for means to understand and control hematopoietic neoplasms of man involves scientists of many disciplines. A large amount of information has been accumulated about leukemias and lymphomas of man, chickens, and some species of rodents. Relatively less is known of these diseases in other animals.

This Symposium was planned to compile information about the characteristics of these diseases in as many animals as possible by calling on the special knowledge and experience of veterinary and medical pathologists from many institutions of the United States and abroad. The Armed Forces Institute of Pathology drew upon the resources of its Veterinary Pathology, Comparative Pathology, and Lymphatic Tumor and Hematopoietic Registries.

Because of a traditional and continuing interest in comparative pathology, the Armed Forces Institute of Pathology was proud to host this meeting and to share the responsibility of publication of this Monograph.

Bruce H. Smith, *Captain, MC, USN*
The Director
Armed Forces Institute of Pathology

Perspectives in Comparative Oncology[1]

HAROLD L. STEWART, *Laboratory of Pathology, National Cancer Institute,*[2] *Bethesda, Maryland 20014*

In 1802, now 166 years ago, a *Medical Committee of the Society for Investigating the Nature and Cure of Cancer* was established in Edinburgh. The Committee's aim was: ". . . the acquisition of knowledge on a subject of which, it may be said, we are even at this time totally ignorant." Not much different than today, you see.

I continue to quote from the Committee's report: "In order to form a basis of inquiry, in which the nature and cure of cancer, it is presumed, may be pursued with all the advantages of reason and experience, the Medical Committee very early drew out, and distributed (*13*) *queries*, for the consideration not only of the corresponding members, but of all medical men, to whom opportunities of answering them might, by study or by accident occur."

All 13 of these queries are as canny as might be expected from Scots of the early 19th Century. Query #10 is pertinent to this meeting, so I shall read it, along with a more lengthy explanation of its aims and purposes as stated by the Medical Committee:

"Query 10th—Are brute creatures subject to any disease resembling cancer in the human body?"

"It is not at present known whether brute creatures are subject to cancer, though some of their diseases have a very suspicious appearance. When this question is decided, we may inquire what class of animals is chiefly subject to cancer; the wild or the domesticated; the carnivorous or the graminivorus; those which do, or those which do not, chew the cud. This investigation may lead to much philosophical amusement and useful information; particularly it may teach us how far the prevalence or frequency of cancer may depend upon the manners and habits of life. As establishments are now formed for the reception of several kinds of animals, and as the treatment of their diseases has at length fallen under the care of scientific men, it is hoped that the information here required may be readily obtained. If animals which live only on herbs, and never drink any other liquid than water, prove to be the least, or not at all, subject to cancer, such proof may, in many cases, become a guide in practice."

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

That reference to water as the sole beverage of "the brute creatures," in the last sentence, set me to wondering if some of these Scottish physicians had guilt feelings about Scotland's large consumption of whiskey, or "usquebaugh" as they then called it, which translated means "water of life." Perhaps these Scots, who may have been drinking less water and more whiskey, were fearful, God forbid, that whiskey might turn out to be a potent carcinogen. If this had proven so, it might have ruined the largest and finest export industry of Scotland. Besides, the joy of all mankind would have been considerably decelerated over the period of these 166 years.

This old, yet up to date, 10th query about cancer in *brute creatures* yielded information, but only slowly, for it was not until 50 years later in 1858–59, now 100 years ago, that Leblanc in France reviewed and appraised case reports of tumors in the horse, mule, donkey, cow, dog, cat, and pig, and emphasized the importance of histologic examination in the diagnosis of tumors of animals. The recognition that tumors do occur in lower animals was, he pointed out, of fundamental importance to the comparative pathologic study of cancer.

At the turn of the century, McFadyean, a Scot, then earning his living working in London, and Sticker, from Germany, published extensive data on tumors in domestic animals; about this time the British Imperial Cancer Research Fund began an investigation of the distribution of tumors thoughout the animal kingdom. By 1904, Bashford and Murray, of the Imperial Cancer Research Fund, could state definitely that cancer occurs in wild as well as in domestic animals; they believed further that the disease in animals was essentially the same as the disease in man, and predicted that cancer would probably be found to occur throughout the animal phyla.

Apart from Nowinsky's work in 1876 on the transplantation, from one animal to another, of the venereal tumor of the dog, it was not until around the turn of the century that the transplantation of tumors in rodents got well under way. As a result of the systematic charting of the growth of transplanted tumors, it was recognized in about 1904 that mice, in which transplanted tumors had regressed, were immune to subsequent transplants of the same tumor and sometimes to other tumors as well. According to the late Dr. Clowes, this basic discovery of an immunologic factor in cancer in mice was principally responsible for the expansion of cancer research throughout the world in the early years of this century.

Then came the discoveries in 1908 and 1910, respectively, of the viral transmission of chicken leukemia by Ellerman and Bang and of chicken sarcoma #1 by Rous. This was followed in the 1930's by the work of

Sanarelli, Shope, Bittner, Korteweg, and others on viral cancers and related conditions in laboratory and wild animals. Even with these epochal discoveries of virus tumors in animals on up through the 1930's, no university at that time thought it worthwhile to establish a chair of Virology in any Medical School in this country, showing how far education lags behind research.

The later work on virus-induced neoplasms and, in particular, on virus-induced hematopoietic neoplasms, initiated by the discoveries of Gross, Stewart, Evans, Friend, Graffi, Moloney, Rauscher, and others has led to studies on mechanisms and pathogenesis of viral carcinogenesis that have revealed biologic processes not even suspected before. Thus all of our knowledge of viral carcinogenesis has derived from comparative oncology studies, because to date viruses have not been shown to cause cancer in man.

All of the known agents that *do cause cancer in man,* be they physical or chemical, were discovered from studies *in man.* I can't recollect a single causative agent of cancer in man that was discovered from work in comparative oncology—not even cigarette smoking and lung cancer. So studies in man are important also. Nevertheless for the elucidation of the mechanisms and pathogenesis of carcinogenesis by physical and chemical agents, recourse must be had to the lower animals, or the brute creatures, as that very modern-thinking Scottish Committee of 1802 designated them. And so, much knowledge about the action of chemical and physical carcinogens and the discovery of many new carcinogens for animals have derived from studies of comparative oncology.

In September 1966, the National Advisory Cancer Council directed a National Cancer Institute staff team to survey the field of chemical carcinogenesis to assess needs for support. As I said earlier, since physical and chemical agents are the only known causes of cancer in man, the emphasis of this survey was on carcinogenic studies with such agents. As one might suspect, many recommendations dealt with support and expansion of laboratory work to develop a better understanding of the mechanisms of action of chemical carcinogens and factors that influence these processes. Included among them were studies of immunology, regulation of growth and differentiation, the role of hormones, carcinogens in relation to metabolic processes, and the early events in carcinogenesis. Especially emphasized were the conversion of target cells to cancer, macromolecular synthesis, and ultrastructural changes. To carry out these programs, detailed suggestions were made with regard to animal facilities, genetically defined animals, gnotobiotic animals, and provisions for monitoring animal colonies with respect to diets, infections, hormones, genetic drift, and the like. The survey team pointed out that, whereas mice, rats, fish, and avian species are currently in use, other species need to be investigated, particularly carnivora, such as ferrets. If carcinogenicity and teratogenicity are to be considered together, then species with short gestation periods and different types of placenta, such as the opossum and the armadillo, should be investigated.

I thought this survey team was very wise to stress the contribution to our knowledge of cancer that might be expected from epizootiologic and epidemiologic studies of animal populations. Very useful, they pointed out, would be systematic surveys of indigenous animal populations in this and other countries to detect and describe the sites and types of neoplasms and to elicit geographic variations. Systematic natural-history investigations would contribute data on neoplastic entities in a wide variety of vertebrate and invertebrate species. Variability of cancer susceptibility has been shown in different breeds of dogs, such as a high risk in boxers and the frequency of bone tumors as it is correlated with length of bone.

The expansion of systematic studies of neoplasms in the relatively short-lived household pets, dogs, cats, canaries, and parakeets, that share man's environment is greatly to be desired. Our distinguished Chairman, Professor Cotchin, has contributed significant observations in this area of investigation. It must be remembered that the sex distribution of neoplasms in the canine and feline species might critically reflect exposure to exogenous carcinogens in relation to habits, customs, and instincts. While the habitat of the female of these species is pretty much restricted to the property of her master, the male lives in a less restrictive environment. His biological urges and the keenness of his scent may impel him over a considerable expanse of territory beyond his master's property in the pursuit of the object of his desire. In the course of his peregrinations he takes a careful, minute, and detailed nasal inventory of the terrain he traverses. How many carcinogens does he inhale during these expeditions? Here then is a built-in experiment of nature to be taken advantage of by cancerologists.

To help promote animal studies, the survey team further recommended extension and expansion of already existing registries for tumors and the establishment of new ones to accession examples of tumors in species not previously studied. There are now many large collections of animal tumors on the European continent, in Britain, and in the United States. The World Health Organization has a group working on a code and definitions of cancer types of domestic animals. There need to be additional collections of tumors, particularly of uncommon species, in zoo animals, fishes, amphibians, reptiles, and invertebrates to permit evaluation of their usefulness in experimental carcinogenesis. In the interest of better dissemination of available information in comparative oncology, the survey team recommended the production of atlases on the pathology of spontaneous and induced tumors in animals.

While there are many similarities, there are still many differences that we cannot explain between cancers in human beings and in the lower animals. Why, for example, is cancer so much more frequent in the mammary gland of certain strains of mice than in women, and why is it almost unknown in the udder of cows? Can these differences be ascribed wholly to the mammary tumor agent and hormonal constitution? Why in women does mammary cancer metastasize earliest to the regional lymph nodes, whereas in mice a lymph node resists secondary deposits even though surrounded by and embedded in the tissue of a mammary cancer that has already metastasized to lung? Does a species exist whose mammary cancer duplicates closely enough, histologically and biologically, mammary cancer in the human female, so that it might serve as a useful model for studies in comparative mammary oncology? Answers to these and many other enigmas presented by comparative oncology must be sought. Such answers would not only advance basic research but would place at our disposal knowledge calculated to contribute to the control of cancer in man.

Neoplasms of Blood Cell Origin in Poikilothermic Animals—A Review [1]

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SUMMARY

For the "lower," cold-blooded animals, knowledge of the morphology of hematopoietic, or reticular, neoplasms is extremely limited, especially at high-resolution levels. This is true in part because of difficulties in the biologic definition and recognition of these neoplasms in animals that differ so greatly from the mammals. In reptiles, hematopoietic neoplasms have not been reported. Sarcomas have been described in snakes and turtles inoculated with avian sarcoma virus (Schmidt-Ruppin strain), suggesting that experimental studies with fowl and murine leukemia viruses should be conducted, using reptilian recipients. In amphibians, conditions designated lymphosarcoma have been described and investigated experimentally. These conditions are clearly transmissible, but on the basis of existing morphologic and experimental evidence, their homology with lymphoid or other reticular neoplasms of mammals is considered questionable. The possibility that these transmissible diseases are infectious granulomas needs to be excluded. In the bony fishes, relatively large numbers of hematopoietic neoplasms have been reported, and on morphologic grounds there seems little question that many of these resemble hematopoietic neoplasms of warm-blooded vertebrates. However, no transplantation or induction system has been developed and put into laboratory use. Most of these neoplasms have been classified as lymphosarcomas, and some have been associated with a leukemic blood picture. The northern pike (*Esox lucius*) seems to offer unusual opportunity for experimental studies, as lymphosarcoma in this species has been reported in epidemic proportions in Ireland. The neoplasms are discovered most frequently through their involvement of the mucous membranes of the oral cavity or the skin of the trunk. Cytologically, these neoplasms appear to correspond to undifferentiated hematopoietic stem cell sarcomas of mammals, although there are morphological features suggesting they may be derived from the plasma cell series, or even from erythroblasts. Salmonid fish and Mexican charicins have also been reported to develop lymphomas, with the thymus implicated as the site of origin, although the mesonephros is also heavily involved. The cell type has been classified as an immature lymphoid cell. In molluscs, lesions possibly comparable to hematopoietic neoplasms of higher animals have recently been described in a small number of oysters of the species *Crassostrea virginica* and *Crassostrea gigas*. Further studies on additional examples will be required before the nature of

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

these unique and interesting lesions can be established. Among insects, lymphosarcoma, leukosarcoma, and leukemia have been reported, but the true nature of these conditions remains in doubt. The so-called lymphosarcomas of *Drosophila* are certainly not of lymphoid cell origin, as insects do not have lymphocytes by definitions based on mammalian hematology. The lesions are probably hemocyte reactions analogous to granulomas, although genetic factors play a role. A proliferative hemocyte response in Mayfly nymphs parasitized by chironomid larvae deserves reinvestigation. It may be neoplastic or it may be comparable to the blastogenic response of mammalian lymphocytes to phytohemagglutinins and other mitogens. Among the invertebrates, morphologic criteria for identification of hematopoietic neoplasms have not yet been delineated. For this reason, work in this segment of comparative oncology remains in a descriptive phase, awaiting the establishment of correlations between biological and morphological data.—Nat Cancer Inst Monogr 32: 7–28, 1969.

WITHIN HUGE provinces of the animal kingdom, next to nothing is known concerning the existence, let alone the morphology, of neoplasms arising from hematopoietic cells. These dark provinces lie almost entirely within the realms of the cold-blooded animals, vertebrate as well as invertebrate. Much more information is available for the vertebrates than for the invertebrates, but this does little to reduce the size of *terra incognita,* since invertebrate metazoans comprise over 95% of all existing species (*1*, *2*).

While some metazoan invertebrates have no blood vascular systems traveled by migratory cells, it *cannot* be contended that such animals should be excluded from comparative hematologic studies. Metchnikoff's classical experiments (*3*) argue that even the most primitive metazoans have fixed mesenchymal cells that may be mobilized as wandering phagocytes representing the phylogenetic precursors of blood cells, just as fixed, undifferentiated mesenchymal cells in mammalian embryos represent the ontogenetic precursors of circulating blood cells. This review therefore covers neoplasia of the reticular tissues defined in the broad sense outlined by Marshall (*4*) and applied by Dunn (*5*) in her studies of hematopoietic neoplasms of rodents.

REPTILIA

Primitive reptiles are thought to have been in the direct line of descent of primitive mammals, and birds are believed to have branched from the primitive reptile line earlier than the primitive mammals. It is interesting then that no leukemias, leukoses, or reticular neoplasms of any sort have been reported in reptiles, while avian leukoses and reticular neoplasms of mammals are only too plentiful. Plausibly the absence of positive information concerning the reptiles may be related to inadequate investigations of disease in this class. This seems the more probable in view of Svet-

Moldavsky's recent report (*6*) that the Schmidt-Ruppin strain of Rous sarcoma virus produced growths in one species of serpent (*Erix tataricus*) and in one species of turtle (*Testudo horsfieldi*). He designated these growths as sarcomas composed of polygonal, spindle-shaped, and round cells forming a histological pattern similar to that in the sarcomas induced by the same virus in fowl. The precise identity of the cells that give rise to Rous sarcomas in birds and their relation to cells of the reticular (reticuloendothelial) system remain unclear, as does the cell type of origin of the reptilian tumors described by Svet-Moldavsky. However, Svet-Moldavsky's finding, coupled with recognition that the Rous and avian leukoses viruses are closely related, should stimulate investigators to study the effects of fowl leukoses viruses and even of murine leukemia viruses in reptiles and amphibians.

The effects of snake venoms on the murine leukemogenic viruses have been investigated. Okabe, Sugimura, and Kasuga (*7*) reported in 1964 that cobra venom inactivated the Friend virus. Even boiled venom was effective, and because phospholipase A was the only enzyme with activity remaining after boiling, it was concluded that the Friend virus must contain a phospholipid component. Later it was shown with the Rauscher virus that phospholipase C acted on the envelope of cell membrane around the virion rather than on the virion itself (*8, 9*). Complicating things still further, Padgett and Levine (*10*) reported herpesvirus-like structures in venom-treated Rauscher virus preparations, and thought these were produced by the action of enzymes on the Rauscher virus. This interpretation was controverted by a recent report of Monroe *et al.* (*11*), who found that cobra and banded krait venoms commonly contain large numbers of herpesvirus particles. Their presence is not relevant to the action of venom on Rauscher virus, but it is interesting that venom produces a characteristic honeycomb pattern in the cell membrane envelopes around Rauscher virus particles (*11*). This phenomenon probably relates to the finding of Friedman and Pastan (*12*) who demonstrated an antiviral replication effect of a phospholipase (phospholipase C from *Clostridium perfringens*) acting on the (RNA) Semliki Forest virus-chick fibroblast system. This antiviral action depended on the enzymatic modification of cell membrane activity necessary for viral RNA replication.

This commentary does not imply that venomous snakes should be resistant to RNA viruses (such as fowl leukosis and murine leukemia viruses) simply because phospholipase is present in their venom.

AMPHIBIA

For this group of cold-blooded tetrapods the reviewer believes the existing morphologic and experimental evidence is inadequate to establish, beyond question, that hemic neoplasms have been observed. Nevertheless, there is a series of reports describing lymphosarcomas in the African claw-footed toad, *Xenopus laevis*, and reporting the transmissibility of these neoplasms by cell-free homogenates and filtrates (*13–30*).[3]

In 1962 Balls (*13*) reported 5 examples of lymphosarcoma, one accompanied by lymphocytic leukemia, in toads of the genus *Xenopus*. Two of these were in *Xenopus laevis*, 2 were in *Xenopus fraseri*, and 1 was in a toad produced by transplanting a nucleus from a blastula cell of *X. laevis victorianus* to an enucleated ovum of *X. laevis laevis*. The lesions in these animals involved chiefly the liver, spleen, and kidneys, and in some instances the muscles of the head. Some of the photomicrographs accompanying the report are reasonably consistent with morphological concepts of lymphomas in other vertebrates, but all were at low magnifications and fine cytologic detail was not presented. The lesions illustrated in Balls' figures 2, 13, and 14 (*13*) are densely cellular and are apparently composed of monomorphic, lymphoid cell types. On the other hand, lesions illustrated in figures 3, 6, 7, 10, and 11 are strongly suggestive of granulomatous reaction. The cells composing the nodules appear to have a relatively voluminous cytoplasm characteristic of histiocytes or epithelioid cells, and the centers of some nodules are necrotic, as the central parts of infectious granulomas often are.

In subsequent studies, Balls (*18*) reported the "transfer" of one of the original growths, and Ruben and Balls reported induction of lymphoid neoplasms similar to those described in the 1962 report (*21*). Transformation of recipients' lymphoid cells to lymphosarcoma was reported after tissue transplantation across genus and species lines, for example, to *Rana pipiens* (*20*). Induction was also reported (*15*) after benzpyrene was injected into the dorsal lymph sac or after benzpyrene crystals were placed in the abdominal cavity. In the latter experiments, lesions also appeared in the wounds through which the hydrocarbon was introduced. To this reviewer, the lesions illustrated resembled granulomas more than lymphosarcomas. Balls (*14*) also reported induction of lymphosarcoma in *X. laevis* by means of methylcholanthrene. In 1964, Balls and Ruben (*16*) reviewed the chemical induction of neoplasms in amphibia, including the earlier work of Leone (*31*) in 1957, in which tumors were reported in 22 of 47 newts (*Triturus cristatus*) treated with methylcholanthrene. Leone thought these lesions should be classified as lymphosarcomas, but his illustrations 5, 6, and 7 show features typical of granulomas, and closely similar to those illustrated in Balls' figures 3, 6, 7, 10, and 11 (*13*).

Later in 1967 (*29, 30*) it was reported that lymphosarcomas could be induced by cell-free extracts of *Xenopus* lymphosarcomas, and a viral etiology was postulated. Studies of lymphosarcomas appearing after homologous and heterologous grafting of the lesions were interpreted as proof that induction rather than transplantation of lymphosarcomas occurred after heterologous and homologous grafting of lymphosarcomatous tissue. Balls and Ruben (*26*) also described induction of lymphosarcoma by normal tissue implants, and a hypothesis was developed that homograft reaction initiates a change of latent virus to active virus, which in turn induces lymphosarcoma in the graft recipient.

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[3] *Note added in proof:* L. E. DeLanney and M. K. Blackler have recently reported studies of a transplantable lymphosarcoma in the Mexican axolotl, *Siredon mexicanum*. Their paper, entitled "Acceptance and regression of a strain-specific lymphosarcoma in Mexican axolotls," was presented at the symposium on Biology of Amphibian Tumors. Oct. 28–30, 1968, and will be published by Springer-Verlag, New York and Berlin, under editorship of M. Mizell.

In their most recent papers (*29*, *30*), Balls and Ruben reported induction of lymphosarcomas in *Xenopus* inoculated with filtrates of lymphosarcoma homogenate through filters of 100 mμ pore size. However, they acknowledged difficulty in repeating the 100 mμ filter experiment (*29*), apparently because of adsorption of the agent by the filters. In one experiment, 9 of 10 control animals kept in the same tank with filtrate-inoculated animals developed the disease. Therefore there is little question that the disease is transmissible, but whether it is a neoplastic disease, and in particular a lymphosarcoma, remains unsettled.

Recently Dr. Ruben submitted (Accession No. 109, Registry of Tumors of Lower Animals, Smithsonian Institution) histological sections and a paraffin-embedded block of tissue (liver) from a second example of a transmissible disease in *Xenopus*, also under consideration as a lymphosarcoma and differing, according to Dr. Ruben (*32*), only slightly in its morphology from the serially transmitted disease previously reported. The organs chiefly affected in this second transmissible disease were liver, spleen, kidney, and serosal surfaces of the viscera, although the lesion in the original host had been on the snout. On the basis of this material, I found it difficult to interpret the lesions as other than infectious granuloma. Some of the lesions were quite extensive and destructive of liver parenchyma, but they were composed mainly of histiocytic cells, often forming discrete foci adjacent to vessels and sometimes having a narrow areola of small lymphoid cells around the periphery. Central necrosis was present in some of the larger foci, and multinucleated giant cells of the Langhans type were occasionally present. Most significant, however, was the presence of intracellular acid-fast bacilli in some of the histiocytes in the lesions. Usually only one or two such organisms were present in a given cell, but a large proportion of the lesions contained them. Dr. Ruben informed me that *Xenopus* Lymphosarcoma I lesions also contained acid-fast bacilli, but that he has been unable to reproduce the disease by inoculating toads with cultures of these organisms. He has therefore considered them to be secondary infectious agents not causally related to the disease.

While the lymphosarcomatous nature of Lymphosarcoma I as studied by Balls and Ruben may eventually be substantiated, granulomatous reactions have often been mistaken for neoplasms, because of their ability to produce large, space-occupying masses culminating in death of the host. Such deceptive lesions have also been encountered in mammals. Symeonidis and Emmons (*33*), for example, described a mycotic infection that simulated neoplastic growth in mice. The visceral granuloma of brook trout (*34*) was originally reported as a neoplasm (*35*). As early as 1904, Bashford (*36*) recognized the difficulty of separating granulomatous diseases of fish from neoplasms: "The unequivocal nature of the microscopical preparations of adeno-carcinoma in fish obviates completely the objection that might be raised, were sarcoma (so often with difficulty distinguished from infective granuloma) alone recorded in this class." Dhahliwal and Griffiths (*37*) described a fungal infection in Malayan toads that produced large tumor-like reactions. Machicao (*38*) found leproma-like granulomas in several species of frogs and one species of toad in Bolivia, and demonstrated myriads of nonculturable intracellular acid-fast bacilli in insects on which these amphibians feed. He presumed these insects to be carriers of the infection. Darzins (*39*) described an epizootic of tuberculosis among the Gias (frogs) in Brazil, and isolated the causative organism from the water in which the animals lived.

Inoue and associates (*40-43*) reported transplantable "sarcomas" in the Japanese newt, *Triturus pyrrhogaster*, but in their most recent publication (*43*) they reported finding acid-fast bacilli in the lesions, and showed that cultures of the organisms produced lesions identical to those they had considered neoplastic. Morphologically, these lesions as judged from published photomicrographs are more consistent with granulomatous reactions. The infection was also transmissible to *Xenopus laevis*.

These are merely a few examples of mycobacterial granulomas in amphibians. A large amount of literature exists on the subject of mycobacterial infections in cold-blooded animals, and it was reviewed by Vogel in 1958 (*44*).

The primary lesions first described by Balls (*13*) may have been of different nature than the disease that was serially transmitted. A mycobacterium or even a virus unrelated to the primary disease could have been transferred to the new hosts. One is reminded of the problem that confronted Sternberg (*45*) when he found tubercle bacilli in patients with Hodgkin's disease. Being strongly influenced by Koch's postulates, Sternberg concluded that Hodgkin's disease could not be neoplastic even though he was fully aware of the sarcoma-like aspects of some lesions. If a similar situation existed in *Xenopus laevis*, homogenates of the lesions would produce tuberculosis in recipients, and it might be assumed that the original disease was nothing more than tuberculosis, though it might have been a neoplasm harboring bacteria as contaminants or secondary invaders.

Even if the disease described and transmitted by Balls and co-workers is not lymphosarcoma, but an acid-fast bacillary infection, the disease may be of medical importance. In tropical Africa and Australia, a disease now known as Buruli ulceration (*46, 47*) afflicts many and is apparently caused by an acid-fast organism, *Mycobacterium ulcerans*, which grows well on artificial media at 33°C, but not at 37°C. The anatomic pathology of the disease has been described by Connor and Lunn (*48*). However, the means of transmission and possible animal reservoirs of the organisms have not been discovered. Acid-fast organisms found in cold-blooded animals deserve thorough study to determine whether they may be related to organisms such as that found in the lesions of Buruli ulcer.

PISCES

More information has been collected on neoplasms of blood cell origin in fishes than for any other single class of cold-blooded vertebrates. Curiously, all this information is confined to bony fish (teleosts); there appears to be no positive information about hematopoietic (reticular) neoplasms in cartilaginous fish.

Most of the reticular neoplasms in bony fish have been classified as lymphosarcomas, regardless of whether or not widespread dissemination of lesions occurred, or whether there was evidence of large numbers of cells in the peripheral blood. In bony fish, hematopoiesis takes place mostly in the mesonephros, the thymus, the submucosa of the intestine, and the spleen, with the mesonephric kidney predominating. Lymphosarcomas in most species of fish have been thought to be primary in kidney or thymus, but those of northern pike (*Esox lucius*) appear to be an exception, as the lesions are most prominent, at least, in the subcutaneous tissues or oral submucosa.

In 1948 Schlumberger and Lucké (*49*) collected 20 reported cases of lymphosaromas in bony fishes. These have been included in table 1 with subsequent cases. Mulcahy (*50*) cited Ohlmacher's report (*51*) as the first of a lymphosarcoma in pike. Like the cases described by Mulcahy, this fish had a large externally visible mass which was "embedded" in the trunk muscles. Multiple similar tumors were also present under the visceral and parietal peritoneum. Details of the cellular morphology are not available beyond the fact that the tumor was composed of round cells with large nuclei and scant cytoplasm.

Probably similar to this case and to those reported later by Mulcahy was one in *Esox lucius*, reported by Haddow and Blake (*52*) in 1933. However, it is known only that many subcutaneous and submucosal tumors were present around the fins, in the mouth, and under the gill covers. Advanced autolysis precluded identification of the cell type involved, although it was stated the masses had a highly cellular structure, "apparently lymphatic in origin."

The 12 cases of lymphosarcoma in pike reported by Nigrelli (*53*) appeared to represent an epizootic, as all the fish came from the same tank in the New York Aquarium, and all succumbed during the summer months of the years 1940 and 1941. These cases were described and illustrated in detail. The growths were believed to have originated in the posterior

Table 1.—Hematopoietic neoplasms of fish

Species	Apparent primary site	Other sites involved	Original diagnosis	Author and reference No.	Comments
Northern pike, *Esox lucius*	Region of caudal vertebrae, invading trunk muscles	Peritoneal fat, serosal surfaces, liver, spleen, intestine, and swim bladder	Round cell sarcoma—endothelioma(?)	Ohlmacher (*51*)	Gross and microscopic descriptions indicate close similarity to tumors reported by Haddow and Blake (*52*), Ritchie (*56*), and Mulcahy (*50*).
European flounder, *Platichthys flesus*	Eye or orbit	Eye and orbit only	Lymphosarcoma	Johnstone (*60*)	Tumor may have been retinoblastoma? Choroid and retina were involved, but no hematopoietic organs.
Goldfish, *Carassius auratus*	Kidney	Liver, orbits	Lymphosarcoma	Plehn (*61*)	Original description could not be obtained for review. Data from Schlumberger's summary (*56*).
Herring, *Clupea harengus*	Gonads(?) abdominal peritoneum(?)	None mentioned. Gonads not identifiable, possibly destroyed by tumor	Lymphosarcoma	Johnstone (*68*)	Possibility of seminoma not excluded.
Conger eel, *Conger conger*	Kidney	None	Lymphosarcoma	Williams (*69*)	Said to be cytologically comparable to large-celled lymphosarcomas of man.
Salmon, *Salmo salar*	Kidney	None	Lymphosarcoma	Haddow and Blake (*52*)	Apparently a hematopoietic neoplasm, but cell type not described in detail.
Northern pike, *Esox lucius*	Multiple tumors in subcutis and buccal cavity	No visceral involvement	Lymphosarcoma	Haddow and Blake (*52*)	Distribution of tumors similar to that described by Ritchie (*56*) and Mulcahy (*50*).
Tropical aquarium fish, *Rasbora lateristriata*	Lymphatic tissue near peritoneum (?)	By extension, gill, oral cavity, cranial cavity, auditory apparatus	Lymphosarcoma	Smith *et al.* (*70*)	Cell type described as small lymphocyte. No blood involvement.

Northern pike, *Esox lucius* (12 cases)	Kidney	Liver, spleen, retroperitoneal tissues	Lymphosarcoma	Nigrelli (*53*)	Nodular pattern like follicular lymphoma in spleen. Necrosis and fibrosis in liver.
Mexican characin, *Astyanax mexicanus*	Thymus	Gill arches, skin, testes, viscera, nearly all organs	Lymphosarcoma	Nigrelli (*53*)	Cell type described as small lymphocyte.
Hybrid, *Astyanax mexicanus*, *Anoptichthys jordani*, and *Astyanax mexicanus* (2 cases)	Thymus	Liver, kidney, gills, pancreas, intestine, mesentery, peritoneum, bone, muscle, nerves	Lymphosarcoma	Rasquin and Hafter (*63*)	Responded temporarily to ACTH treatment. Cell type small lymphocyte.
Muskellunge, *Esox masquinongy*	Subcutis, muscle	Liver, spleen, kidney	Lymphosarcoma	Ritchie (*56*)	Distribution of tumors as in pike described by Haddow and Blake (*52*) and Mulcahy (*50*).
Tropical aquarium fish, *Pristella riddlei*	Kidney	Multiple nodules in adomen, muscle invaded	Undifferentiated mesenchymal tumor	Wessing and von Bargen (*71*)	Listed here because possibly a reticulum cell sarcoma. Reported to be transmitted by cell-free filtrates.
Northern pike, *Esox lucius* (many)	Mucous membranes of mouth, or skin	Viscera, kidney, peritoneum, muscle, nerve	Lymphosarcoma	Mulcahy (*50*, *54*, *55*)	Cell type highly undifferentiated reticular cell, possibly differentiating slightly along plasmacytoid line.
Brook trout, *Salvelinus fontinalis* (3 cases)	Thymus (2), kidney and thymus (1)	Liver, spleen, peripheral blood dissemination	Thymic lymphoma	Dunbar (*58*)	Leukemic blood picture, as judged from sections. Cell type an immature lymphocyte.
Splake, *Salvelinus fontinalis* × *Cristivomer namaycush* (2 cases)	Under operculum (thymus?)	Adjacent muscle invaded	Lymphoma, probably thymic in origin	Dunbar (*58*)	Leukemic blood picture, as judged from sections. Cell type an immature lymphocyte.
Silver salmon, *Oncorhynchus kisutch*	Serosal surface, stomach wall (single focus)	None	Reticulum cell sarcoma	Ashley, Halver, and Wellings (*59*)	Fibrosarcoma and mesothelioma not excludable as possibilities.

kidney, although the head kidney is highly developed in this pike. The liver, spleen, and retroperitoneal tissues were involved, presumably metastatically. In the spleen, there was a nodular pattern reminiscent of follicular lymphoma. The centers of the nodules in the liver were sometimes necrotic, with perinodular fibrosis. Mention was not made of a search for acid-fast bacteria or other micro-organisms in these lesions. Cytologically, the cells were characteristic of large lymphoid cells, and numerous mitoses were present. These cases of Nigrelli's differ in the location of the lesions from those described by Ohlmacher (*51*), Haddow and Blake (*52*), and Mulcahy (*50, 54, 55*) in the same species, and also from the lymphosarcomas found by Ritchie (*56*) in the muskellunge, *Esox masquinongy*. Our only information about the latter is taken from Schlumberger's paper (*57*): "About 10 percent of these fish taken from Lake Scugog in the neighborhood of Toronto, Ontario, bear the tumor. The lesions differ from those of the pike described by Nigrelli, in that the tumors arise in the subcutaneous tissue where they invade the trunk musculature. Later, the kidney, spleen, and liver become diffusely enlarged, the result of a lymphoblastic infiltration, and the enlargement is accompanied by lymphatic leukemia. Occasionally the skin lesions heal, and the fish recovers; but the cells appear to be definitely neoplastic, and in most cases the tumors progress until the fish succumbs. Initial studies by Ritchie indicate that the tumor can be transplanted and that it is contagious to muskellunge living in the same water with fish bearing the growth."

Mulcahy (*50, 54, 55*) has encountered more examples of lymphosarcoma in *Esox lucius* inhabiting the lakes and streams of Ireland than have been seen in all varieties of fish by the aggregate of other investigators reporting this disease. However, of the more than 400 instances she recorded during a several-year program of pike eradication in Ireland, only half a dozen were preserved in condition satisfactory for histologic study (*50*). The anatomic sites most frequently involved in her specimens were, in descending order: lower jaw, upper jaw, tongue, skin of trunk, inner side of operculum, pharynx, gill bars, head, stomach, and kidney, with other sites occasionally involved. The overall frequency of tumors in some 310,000 fish examined (mostly by nonscientific inspectors) was about 1 per 800, but in some small samples was as high as 1 per 8 or 1 per 12 fish (*54*). Drawings published by Mulcahy (*50*), depicting the extent of jaw involvement that was often found, may suggest to some that the gross anatomical features of the disease resemble those described for lymphomas of African children (Burkitt's tumor).

Microscopically, the lesions were described by Mulcahy to be composed of cells with ". . . large basophilic round or oval nuclei, averaging 5.25 μ in diameter. A small number of cells with more densely staining nuclei and slightly more cytoplasm, and also some plasma cells were scattered throughout the tumors. Occasionally very large macrophages with much cytoplasm, containing dark-staining granules, were visible. Usually there were many mitoses, but in some tumours there were few."

Dr. Mulcahy submitted sections and tissue blocks of tumors to the Registry of Tumors of Lower Animals at the Smithsonian Institution (RTLA Accession No. 112). A review of these convinced me that the tumors are reticular neoplasms that invade muscle, skin, and nerve. The cell type from which they arose, however, is difficult to establish from limited material. Most of the cells were of strikingly uniform morphology, which is characteristic of many hematopoietic neoplasms. The cells resembled large "blastoid" or stem cells, except that generally only a single, moderately large nucleolus was present per nucleus. The nuclear membranes were moderately coarse, while the nuclear chromatin was distributed in a delicate network. The cytoplasm was extremely basophilic in Giemsa preparations, and also gave a positive reaction with periodic acid-Schiff staining in eccentrically placed juxtanuclear areas. In sections stained by Wilder's silver method for reticulin, very little reticulin was seen within the cell masses, although the cells showed a tendency to adhere in rows to the few strands of reticulin present. To the reviewer, there seems a distinct possibility that the cell line involved is plasmacytic. An erythroblastic origin also seems possible.

The relative abundance of these neoplasms among pike in Ireland should make it possible to classify them more decisively. Electron microscopic studies should be especially helpful, and analysis of the serum proteins is needed to provide evidence on the possibility of plasma cell origin, which is suggested by the strong cytoplasmic basophilia and perhaps by the positive PAS reaction in the cytoplasm.

Lymphosarcomas have been reported also in salmonid fishes (table 1). Haddow and Blake (*52*) reported a case in a 5-year-old female Atlantic salmon, *Salmo salar*. The entire kidney, but no other organ, was involved, and blood studies were not described. The mesonephros, or head kidney, was thought to have been the site of origin as it was the most extensively enlarged, producing occlusion of the hemal arch. Sufficient information to permit precise identification of the tumor cell type was not presented.

Two other reports of reticular neoplasms of salmonids are to be presented at the Symposium on Neoplasms and Related Disorders of Invertebrate and Lower Vertebrate Animals. The report of Dunbar (*58*) includes 3 brook trout (*Salvelinus fontinalis*) and 2 splake (hybrids of *Salvelinus fontinalis* crossed with the lake trout *Cristivomer namaycush*). In all of these fish, the neoplasms were classified as lymphoid and were believed to have arisen in the thymus. Dunbar suggested a genetic predisposition of brook trout to lymphoid neoplasia, and ascribed the appearance of similar tumors in splake to transmission of this genetic trait from the brook trout parent.

A reticulum cell sarcoma in a silver salmon (*Oncorhynchus kisutch*) has been reported by Ashley, Halver, and Wellings (*59*). In this case, the tumor was an encapsulated mass attached to the serosal surface of the anterior wall of the stomach. Microscopic features presented some of the difficulties inherent in distinguishing between reticulum cell sarcoma, fibrosarcoma, and mesothelioma, although the authors did not discuss the last 2 possibilities.

Three of the lymphosarcomas listed in table 1 had the common feature that exophthalmos was noted in the afflicted fish, in two instances as a result of orbital and/or bulbus involvement. In the European flounder, *Platichthys flesus* (*60*), the tumor was reported as lymphosarcoma primary in the orbit, although it seems difficult to be sure this was not a retinoblastoma. In a goldfish, *Carassius auratus*, Plehn (*61*) observed exophthalmos associated with lymphosarcomatous involvement of liver and kidney, but the exophthalmos was evidently caused by orbital edema resulting from renal insufficiency. Incidentally, the tumor illustrated and described by Plehn in a pike [(*61*), pp 424 and 425] was probably a lymphosarcoma, as it originated in the kidney and spread to the trunk muscles. However, the specimen was too autolyzed for accurate histological classification.

In the Mexican characin, *Astyanax mexicanus*, extension was noted from the thymus to the orbits (*53*). In this case, Nigrelli observed that the masses involving the thymus and gill arches were so extensive that they pushed the gill covers out to a position nearly perpendicular to the body axis. Practically all the organs of the host were involved, including the skin, liver, kidney, gill arches, gut, and testes. The cell type was a small lymphocyte, not remarkably atypical. In a subsequent description of 2 similar neoplasms of *Astyanax*, Friedman (*62*) found the average nuclear diameter of the neoplastic lymphoid cells was greater than that of normal thymic lymphocytes, and Rasquin and Hafter (*63*), with a similar example, found a high mitotic index. Friedman (*62*) in 1962 reported tissue culture studies on 2 lymphosarcomas of *Astyanax;* this is the only information available on the behavior of piscine lymphoma cells *in vitro*, but continuous cell lines were not established.

At the time of Rasquin and Hafter's 1951 report (*63*), there were 3 known examples of lymphosarcoma in *Astyanax* and 1 in a hybrid offspring of *Astyanax* crossed with its blind relative, *Anoptichthys jordani.* All were bred in the laboratory of the American Museum of Natural History, including the one reported by Nigrelli (*53*). These 4 "spontaneous" lymphomas were seen among a total of 2,000 fish; 3 were in adult males, and 1 in an adult female.

One of the 2 lymphomas referred to by Rasquin and Rosenbloom (*64*) occurred in a characin kept in total darkness and the other in a characin kept in the light. The effect of light on the hematopoietic organs of characins is a phenomenon of potential value to future investigations. Rasquin and Rosenbloom reported that the thymus undergoes marked involution when characins are kept in the dark. Nigrelli (*53*), in his 1947 report of 1 lymphosarcoma in a characin, included observations on 2 additional characins, which had been kept in darkness. (*See* p 105 and footnote on p 105.) He was inclined to interpret one of these cases as an example in which the thymus was the seat of lymphosarcoma in an early phase of development, and the other as an example of lymphoid hypertrophy involving the thymus, possibly transitional to lymphosarcoma. The 4 cases later referred to by Rasquin and Hafter (*63*) apparently include 2, if not all 3, of the fish described in Nigrelli's earlier report. At any rate, further investigations are needed to determine whether dark-induced thymic involution in characins may be followed by a high incidence of thymic lymphomas. In rats and mice inoculated with murine leukemia viruses, cortical atrophy of the thymus occurs regularly prior to the appearance of lymphoid neoplasms in that organ (*65–67*).

Three other reports listed in table 1 all described growths of lymphoid cell type, though no unique morphological data were provided. In Johnstone's case (*68*) in a herring, *Clupea harengus*, the primary site was probably the subcapsular tissue of the gonad, which is a site of normal hematopoiesis in many bony fish. In Williams' case (*69*) in a conger eel, *Conger conger*, the tumor apparently originated in the hematopoietic tissue of the kidney. The lymphosarcoma reported by Smith *et al.* (*70*) also probably started in the kidney, although during a 2-month observation period the growth was seen to extend cephalad where it involved the gills on one side and caused unilateral outward displacement of the gill cover, like that seen bilaterally in thymic lymphosarcoma of *Astyanax* by Nigrelli (*53*).

In table 1, the lesions reported by Wessing and von Bargen (*71*) are somewhat problematic. The authors classified the disease as an undifferentiated mesenchymal neoplasm. Their figure 7 suggests a resemblance to the histiocytic type of reticulum cell sarcoma in mammals, but other illustrations showing lesions with much necrosis lead one to suspect infectious granuloma. Transmission of the disease to guppies as well as to 3 other species of tropical fish by means of cell-free filtrates was described, but the passage material was subsequently lost through a laboratory accident.

MOLLUSCA

Two reports of possible hematopoietic neoplasms in oysters will appear shortly in another publication (*72*, *73*). These contain the only information now available on hematopoietic neoplasia in molluscs; the authors have kindly allowed me to review their histologic material and the original manuscripts, from which the following information is derived.

Farley (*72*) described 6 specimens, 5 of American oysters, *Crassostrea virginica*, from the Chesapeake Bay, and 1 of the Pacific oyster, *Crassostrea gigas*, from Matsushima Bay, Japan. All 6 had diffuse infiltrations in practically every tissue and organ by cells that apparently were atypical derivatives of hyalin hemocytes, one of the two major types of blood cells in oysters. This cell type morphologically resembles lymphoid cells of vertebrates, while the other type, known as the phagocytic hemocyte, more nearly resembles cells of the monocyte–macrophage–histiocyte group in vertebrates.

In Farley's cases, the size and degree of maturation of the atypical hyalin hemocytes varied from case to case, ranging from small, relatively mature forms to large, blastlike cells. In most of the diseased animals, mitotic figures were numerous in the atypical blood cells. This feature was emphasized, because even in extremely profuse hemocytic reactions to inflammatory and infectious agents, mitotic figures in hemocytes of oysters are extremely rare. In cases where the cell type was the larger, immature form of hyalin hemocyte, there was a marked increase of pyroninophilic material, presumably RNA, in the cytoplasm. Farley found the infiltrations of atypical cells particularly heavy in the gonads, and for this reason and the fact that the cells also had considerable resemblance to primordial germ cells, he considered the possibilities that *a*) the gonads may be the site of hematopoiesis in oysters, or *b*) the abnormal proliferations might represent neoplasms of germ-cell origin, comparable to seminoma or dysgerminoma in mammals. Farley found no evidence of infectious or parasitic disease to account for the greatly increased numbers of hemocytes, and he felt the proliferating cells could not themselves be some species of protozoan infesting the oysters. Edema, emaciation, and mantle recession were given as evidence that the disease in these animals would have had a fatal outcome if allowed to follow its course.

Farley found the 6 cases among some 30,000 oysters that he examined histologically in a survey of oyster diseases. The nature of the disease will require classification through further study, but his report provides a new stimulus for investigations in comparative hematology.

The same is true for the case to be published by Couch (*73*), which concerns a single American oyster, *Crassostrea virginica*, in which focal proliferations of blastoid cells were found in the mantle. These focal cell collections were made up of cells much larger than mature blood cells of oysters, and quite similar to stem cells seen in mammalian neoplasms of undifferentiated hematopoietic cell origin. The walls of hemolymph sinuses were infiltrated by the blastoid cells, and many blastoid cells could also be identified in the hemolymph of the vessels surrounded by the lesions. On the basis of cytologic features and the focal confinement of the abnormal cell populations, this example may perhaps be best compared with the stem-cell type of reticulum cell sarcoma as seen in mammals. Until similar examples in various stages of development can be studied, an accurate classification cannot be made. In this case, unlike that described by Farley, no involvement of gonad was found.

INSECTA

Among insects there are two diseases that must be noted. It is not certain that either has close biologic kinship to any neoplastic condition known among vertebrates.

One of these diseases is a host–parasite relationship studied by Codreanu in the 1930's (*74–76*). His studies culminated in a lengthy thesis, published in 1939 (*77*), in which it was maintained that a condition comparable to leukosarcoma was induced by an insect parasite. The hosts were nymph stages of Mayflies of the genuses *Heptaginia* and *Rhithrogena*, found in the mountain streams of Romania; the parasites were larvae of a chironomid fly, *Symbiocladius rhithrogenae*. Codreanu found that the Mayfly nymphs parasitized by the chironomids developed tremendous increases in the number of hemocytes in their hemoceles. The hemocytes in this situation were described as atypical, being larger than normal and having enlarged nuclei and nucleoli. The cytoplasm of atypical cells was more basophilic than that of normal hemocytes, and both normal and abnormal mitotic figures were said to be numerous. Normally, mitoses in the blood cells in the hemoceles of insects are rare. Codreanu believed that the insertion of the mouth parts or secretion of some substance by the parasite into the hemocele of the host stimulated the hemocyte proliferations. This served the purpose of the parasite, whose diet was the hemocytes. The removal of the parasite was reported not to result in cessation of the hemocytic proliferations, and the hosts went on to die from the blood cell neoplasm.

This is a fascinating account which we hope investigators will some day confirm and extend. In view of current knowledge of the mitogenic action of phytohemagglutinin, pokeweed, and certain antigens, there may be an alternative possibility—that the chironomid parasite produces a material with such mitogenic action that it could account for the hemocyte proliferation observed.

The second disease of interest has a long and complex history that is beyond thorough review here. It is most often referred to as melanoma, or melanotic tumor, although both these terms are objectionable because they imply homology to melanomas of vertebrates, even though the cells involved in the production of pigment are blood cells and even though it has been strongly contended that the lesions have little or nothing in common with neoplasms as they are generally understood (*78–81*). Perhaps the best name yet suggested for them is black-spot disease (*81*).

At any rate, the first examples of this group of conditions were reported by Bridges (*82*) and Stark (*83*), and the genetic factors influencing the disease were worked out in subsequent years by many *Drosophila* geneticists. A concise review of this group of lesions is available in a review of neoplasms of insects by Harshbarger and Taylor (*84*). A complete list of references to the literature on *Drosophila* "melanomas" is also available (*85*).

Difficulties in understanding the significance of these lesions from the standpoint of comparative oncology were created early when Stark (*83*) stated that one type resembles typical lymphosarcoma, taking origin in the blood-forming organ. The blood-forming organ of *Drosophila* has been called a lymph gland, but this is confusing, since insects do not have cells that correspond closely, either morphologically or functionally, to lymphocytes of vertebrates.

Antibodies have not yet been demonstrated in insects, and while it appears to be correct that cells within and migrating from the blood-forming organs take part in producing the black-spot lesions, the lesions themselves do not behave like neoplasms, but are more characteristic of granulomas in higher animals (*78, 81*). It was shown in 1940 by Russell (*86*) that the lesions called "malignant" by Stark were not the cause of death of the animals in which they occurred. Rather, death was caused by occlusion of the gut resulting from a dystrophy of the gut epithelium, which in turn related to a lethal gene in the strain involved. When black-spot lesions were transplanted to hosts lacking the lethal gene, the transplants failed to grow and the hosts did not die. Therefore, the black-spot lesions themselves were neither lethal nor uncontrollable in their growth characteristics. The most detailed morphologic studies of the histogenesis of black-spot disease have generally ended with the conclusion that the pigmented lesions are similar to the encapsulation reaction that hemocytes construct in response to infection, parasites, foreign bodies, or injured tissues. Readers will find the publications of Salt and co-workers (*80, 87–89*) most helpful for descriptions of hemocyte responses to infection and injury. A review by Jones (*90*) provides a guide to the morphology of normal insect hemocytes.

However, occasional new evidence and new concepts in support of the neoplastic nature of black-spot disease have been presented. In 1951, Strunge, Gigante, and Bernhard (*91*) studied 3 strains of *Drosophila* with a high incidence of black-spot disease. Using ordinary light, phase, and electron microscopy, they concluded that a hemopathy of leukemic type was associated with black-spot disease in at least one of these strains. This conclusion was based on finding great increases in the number of hemocytes in the blood, numerous mitoses in the hemocytes, and atypical forms of hemocytes including giant, bizarre cells with abnormal cytoplasmic granules. In the absence of definitive criteria for identifying leukemic cells in *Drosophila,* and in the absence of critical biological data such as transplantability of the cells in question, it seems the investigations of Strunge *et al.* (*91*) require confirmation and extension before their findings can be precisely evaluated.

Ghelelovitch, who has studied black-spot disease for many years, proposed (*92*) that the lesions start out as neoplasms of hemocytes, but that the aberrant cell populations are recognized by normal hemocytes of the host which then successfully encapsulate and eliminate the incipient neoplasms. This concept is almost the exact reverse of the also still-unproved theory of autoimmune origin of hematopoietic neoplasms of mammals (*93*). According to the latter theory, hemic cells reacting to antigens in normal cells of the same organism become converted into neoplastic cells.

Elsewhere in this conference the Chediak-Higashi syndrome of man has been discussed (94). This syndrome has been compared with a genetic syndrome of Aleutian mink and of the beige mouse (*95–98*). In all three, the blood cells as well as the melanocytes and possibly many other cells reflect disorders of granule formation (*97–99*). Since black-spot disease of *Drosophila* also involves blood cells and melanin pigment formation and is to a large degree genetically determined, the question may be asked: Is black-spot disease in *Drosophila* equivalent to the Chediak-Higashi, Aleutian mink, and beige mouse syndromes? Evidence at the moment is inadequate to answer this. Electron microscopic studies (*100*) of the hemocytes in black-spot disease have revealed nothing similar to the phenomenom of cytoplasmic sequestration described by White (*99*) in leukocytes in the Chediak-Higashi syndrome. Furthermore, the hemocytes within the pigmented foci of black-spot disease could not be expected to show abnormalities of the pigment-forming organelles, because they do not carry these organelles, according to Perotti and Bairati (*100*). Melanin is first deposited extracellularly around the clustered hemocytes, apparently as the result of triggering the reaction between tyrosine and tyrosinase that occurs when so-called crystal hemocytes are injured or humorally stimulated. [*See* Rizki and Rizki (*101*) for mechanism of pigment formation in *Drosophila*.] Although the primary disorder in the type of black-spot disease in the *tu* B_3 strain seems to be premature release and clustering of hemocytes from the hemolymph gland (*100*), the crystal hemocytes in this disease should in the future also come under scrutiny at the ultramicroscopic level. The role of the virus-like particles observed in black-spot disease by Perotti and Bairati (*100*) also needs to be clarified, since a viral infection in hosts of variable, genetically determined susceptibility might be adequate to explain much of what has been observed in this disease.

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The Avian Leukosis Complex [1, 2]

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THE AVIAN leukosis complex is a group of hematopoietically oriented viral diseases of the domestic fowl (*Gallus gallus*) which results in major economic losses in the poultry industry. In 1967, in the United States, 21 million birds of 2.3 billion examined at slaughter were condemned because of diseases of this complex. The overall losses were $170,000,000 (*54*).

Most diseases of the complex are disseminated proliferative processes involving cells of the hematopoietic system or their precursors. Those which involve lymphocytic cells are the most prevalent. Solid tumors are included in the leukosis complex, although they appear to be unrelated because causative viruses of most avian solid tumors are members of the same families as those causing disseminated hematopoietic diseases (*5*, *6*, *12*, *34–36*).

Classification of diseases of the complex are frequently revised because of increasing knowledge of etiologic agents (*9*, *14*, *17*, *21*, *48*, *75*). The most recent proposal, which encompasses major etiologic and morphologic concepts, will be followed in this review (*12*) (table 1). While this classification is not strictly comparable to that most recently proposed for human hematopoietic neoplasms (*69*), which is necessarily based entirely on morphology, some comparisons between avian and mammalian counterparts can be made. Differences in location and function of avian and mammalian lymphocytes complicate comparative studies. Birds have no lymph nodal systems; rather, aggregates of lymphocytes are diffused throughout the body. In contrast to mammals, the avian lymphocyte functions as the dominant cell in reactions to injury and infection, although avian heterophils, which resemble mammalian neutrophils, are present in blood and bone marrow. The avian lymphocyte is considered to be unstable (*32*).

Of naturally occurring neoplasms of the chicken, lymphoid leukosis and Marek's disease predominate. Unlike comparable diseases of man and

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Table 1.—Avian leukosis complex (*12*)

I. LEUKOSIS-SARCOMA GROUP—RNA, Myxovirus		
Lymphoid leukosis (visceral lymphomatosis, lymphomas, big liver disease)		
Erythroblastosis (erythroleukosis)		
Myeloblastosis (myeloid leukosis, leukemia)		
Myelocytomatosis		
Fibrosarcoma, endothelioma, hemangioma, nephroma		
Osteopetrosis		
II. MAREK'S DISEASE—DNA, Herpesvirus		
Classical Acute	All lymphoid	Neural, ocular, visceral lymphomatosis, fowl paralysis, gray eye, etc.
III. UNCLASSIFIED		
Reticuloendotheliosis		

other animals, spontaneous occurrence of these diseases reaches epizootic proportions, and in some areas these diseases are considered to be enzootic.

I. LEUKOSIS-SARCOMA GROUP

Lymphoid Leukosis

Lymphoid leukosis (*49*) was probably observed as early as 1896 by Caparini (*15*, *60*). Ellermann and Bang (*28*) first reported transmission of the disease by cell-free filtrates, probably by an RNA myxovirus, represented today by the prototype RPL12 (*60*).

Lymphoid leukosis is a disease of mature birds. The liver and spleen are usually involved although no organ is exempt; however, brain involvement is rare. Lymphomas are usually multiple and affected organs are usually enlarged (*49*) (fig. 1).

The pathogenesis of this disease has been worked out experimentally. The virus first attacks cells of the follicles of the bursa of Fabricius (*18*). If the bursa is removed at the time of hatching, the disease fails to develop. This observation led to the concept of the so-called "bursal dependent disease" (*65*). The infiltrating cells are large lymphocytic types, sometimes referred to as hemocytoblasts (*18*), which are producers of γ-globulins (*22*, *39*, *62*). In normal birds, hemocytoblasts are seeded in other organs where they are apparently functional and perhaps replace those of the bursa of Fabricius which atrophies at about 10 weeks of age (*38*). In a virus-infected bird, these cells form a tumor in the bursa which metastasizes primarily to the liver and spleen; the infiltrates can be recognized as lymphomas. The neoplastic proliferation in the bursa occurs within the follicles. The interfollicular tissue is not involved, in contrast to Marek's disease in which lymphomatous changes often occur in the interfollicular tissue at the expense of the follicles.

Lymphoid leukosis often involves the bone marrow and other organs. These appear to be metastases; the disease is not primarily one of the hematopoietic organs. Although leukemia has been reported (*26, 37*), it is rare and probably occurs as a result of a lymphoma in an intravascular position constantly pouring cells into the circulation.

Both vertical transmission of virus through the egg and horizontal transmission among chicks occur, but the carrier hen is the chief vehicle. Elimination of carrier hens can prevent transmission of the disease.

Erythroblastosis

The viral etiology of this leukemia (*1, 3, 23, 24, 28–31, 51, 66–68*) is well established. The RPL12 virus is known to be able to produce this disease (*35*). Inoculating relatively large amounts of virus into a newly hatched chick will result in death from erythroblastosis in a week or two. Delay in inoculation for 1 week with reduced amounts of virus results in development of lymphoid leukosis after several months (*13*).

Erythroblastosis is characterized by an enlarged, bright-red liver and splenomegaly (*49*); because the bone marrow of a young normal bird is deep red, bone marrow involvement is difficult to evaluate by gross examination in birds of younger ages. The virus first attacks the sinusoidal cells of the bone marrow, which appear to be endothelial cells, but which have been variously designated as lymphoid erythroblasts (*33, 37*). In a matter of hours these cells begin pouring masses of erythroblasts into the peripheral blood; they may number as high as 200,000/mm^3 (fig. 2). The characteristic architectural pattern of the avian bone marrow, with sharply demarcated islands of intrasinusoidal erythrocytic hematopoiesis and intersinusoidal granulocytic hematopoiesis, is lost (*46, 74*). The marrow is overrun by non-hemoglobin-bearing erythroblasts. The sinusoids of liver and spleen become filled with the immature cells, resulting in a state known as leukostasis (*32*) (fig. 3).

Erythroblastosis, a rare disease in the field, is primarily a laboratory phenomenon. It resembles erythroleukemia (*20*) of man. Ellermann believed that it resembled pernicious anemia of man (*27*).

Myeloblastosis

Myeloblastosis (*23–25, 30, 52, 53*) is also referred to in the older literature as a viral disease (*28*). In 1941 (*40*), it was definitely established as a component of the complex now known to be caused by the RNA myxoviruses. If viruses of this group are inoculated into chickens a few days of age, the intersinusoidal spaces of bone marrow fill with myeloblasts in a few days, overrun the erythrocytopoietic elements, and begin to proliferate in the periportal areas of the liver and the splenic sinusoids. The predominantly nongranulocytic myeloblast produces a striking leukemia with more than 1,000,000/mm^3 leukocytes in peripheral blood (figs. 4 and 5).

If inoculation of the virus is delayed beyond approximately 8 weeks, the bursa of Fabricius acts as an antagonist and the disease does not develop, in contrast to lymphoid leukosis (*2*).

Myeloblastosis, uncommon in the field, is also primarily a laboratory phenomenon.

Myelocytomatosis

Myelocytomatosis (*49*, *57*), a nonleukemic myeloproliferative disease, may arise in organs other than bone marrow. The distribution of infiltrates is similar to that of lymphoid leukosis. This disease is also caused by a multipotent virus. Masses of myelocytes form small (up to 1 cm), soft, white tumors in all organs including the bone marrow. As in most avian hematopoietic disorders, the cells have an affinity for vascular structures. Histologic examination reveals masses of mononuclear granulocytes with brightly eosinophilic granules (fig. 6).

Myelocytomatosis occurs sporadically in adult birds.

Fibrosarcoma, Endothelioma, Hemangioma, Nephroma

The solid avian neoplasms represent a wide spectrum of cell types which must be included in a discussion of hematopoietic disorders because of their common etiologies. Fibrosarcoma (*72*), endothelioma (*37*), hemangioma (*36*), and nephroma (*16*, *41*, *45*) are examples of solid tumors which can be caused by the RPL12 virus. Of all solid tumors of the fowl, only the ovarian carcinoma, the astrocytoma, and perhaps the melanoma are not known to be caused by viruses of this group. A solid tumor frequently found in field cases is the avian fibrosarcoma; the Rous sarcoma (*72*) is an example. These neoplasms are of interest because of the number of strains of causal virus, some of which adapt to heterologous transplantation. Rous sarcoma viruses are defective in that they lack a protein coat which must be acquired from a virus of the RPL12 type to permit viral replication and production of neoplasms. Knowledge from studies of these neoplasms and their causative viruses has resulted in identification of the so-called resistance-inducing factor (RIF) which can be used to test laying hens for the presence of viruses of the RPL12 type (*73*).

The Rous sarcoma is usually a solid fibroma or fibrosarcoma when it occurs in dense tissues, but is a myxofibroma when it occurs in such sites as body cavities. In these sites it produces large amounts of mucin. It will sometimes regress completely, probably indicating successful immunologic defense by the host.

One of the commonly observed solid tumors is the hemangioma, usually of the liver, although the lungs and skin are also common sites. Hemangiomas seem to arise in a multicentric fashion rather than by metastases.

The nephromas or nephroblastomas are similar morphologically to human embryonal nephromas. In the bird, they are more frequent in adults (*41*), in contrast to man in which they are more frequent in children. The avian nephromas never metastasize. They are of viral origin and may be transplanted.

Osteopetrosis

Osteopetrosis or marble bone is a progressive diaphyseal dysplasia usually of long bones, which is also caused by RPL12 virus (*8*, *44*, *50*, *51*). The result of the proliferative process involving disordered new bone formation is a markedly enlarged bone with little marrow cavity. The disease occurs chiefly in males. Although proliferative in nature, it is probably not a true neoplasm.

II. MAREK'S DISEASE

Marek's disease (neurolymphomatosis gallinarum, range or fowl paralysis) (*4*, *56*, *63*, *64*), first described as a polyneuritis (*56*), also has many features of a neoplastic process. The disease is chiefly one of growing birds and is characterized by multiple neoplasms of lymphoid cells that are difficult to differentiate from those of lymphomatosis.

In the classic disease, the central nervous system is the site of a diffuse nonpurulent encephalomyeloradiculitis (*71*), which, in the brain, rarely progresses to become neoplastic (fig. 7). In peripheral nerves, an inflammatory lesion, characterized by edema and a diffuse infiltration of lymphocytes, histiocytes, and plasma cells is believed to precede the formation of diffuse lymphomas of these structures (*10*, *80*). The larger nerves, as the sciatic, and dorsal root ganglia are common sites of inflammatory and proliferative lesions which may cause grossly visible enlargements of nerves and clinical paralysis (*77*) (figs. 8 and 9). There may be lymphomas in viscera and muscles, although neoplastic proliferations are not seen in all affected birds.

In the acute form of the disease, ovaries, lungs, and hematopoietic tissue, predominantly the bone marrow, are overrun by lymphoblastic cells, resulting in a frank lymphosarcoma or lymphomatosis. The intrasinusoidal spaces of bone marrow are reduced, but the animal usually dies before obvious anemia or leukemia develops. The contradictory term "aleukemic leukemia" probably applies in this situation.

Recent reports of electron microscopic examinations of cell cultures infected with cells from birds with Marek's disease describe particles of a size and nuclear location which suggest DNA viruses, specifically those of the herpes group (*10, 59, 82*). However, intranuclear inclusion bodies characteristic of herpes infections have never been seen in cells comprising the lymphoproliferative lesions. The agent is apparently strongly cell-associated; to date no one has succeeded in separating the agent from the cell, although the disease can be readily transmitted by inoculation of whole blood from affected chickens.

Of considerable interest is that some birds do apparently recover from Marek's disease, a fact substantiated by the incidence of histologic lesions in a flock compared to the morbidity and mortality (*42*).

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Reticuloendotheliosis (T virus) (*61, 79*) and a similar disease caused by the strongly cell-associated JMV agent (*76, 78*) may eventually be included in the diseases of the avian leukosis complex. Additional information concerning the nature of these diseases and their causative agents will be required to properly classify them.

DISEASES OF THE AVIAN LEUKOSIS COMPLEX IN OTHER AVIAN SPECIES

Diseases of the avian leukosis complex are not the problem in other species of birds as in the chicken. It must be emphasized that other species are not ordinarily subjected to the same intensive husbandry practices as the domestic fowl. Large numbers and concentrations of birds and bloodlines play a role in producing high incidences observed in chickens. Maybe, if other birds were subjected to similar practices, diseases of the avian leukosis complex would reach the same alarming proportions.

Lymphoid leukosis is known to occur in turkeys (*Meleagris gallopavo*) (*7, 55*), and it basically resembles the disease in chickens. Osteopetrosis has been produced experimentally (*43*) in this species, although it was a slow process requiring up to 1 year.

The Japanese quail (*Cortunix cortunix japonica*) is definitely susceptible, and both lymphoid leukosis and Marek's disease have been reported (*81*). When the BAI-A strain of virus, which produces myeloblastosis in chickens, was inoculated into newly hatched quail, a series of solid tumors, as nephroblastoma, lymphoma, and rhabdomyosarcoma, were produced but, oddly, no myeloblastic leukemia was produced (*58*).

The disease is less well established in other birds. A histologic description of Marek's disease in a ring-necked pheasant (*Phasianus torquatus*) adds another species (*47*), as does a similar report in a Muscovy duck (*Cairina moschata*) (*19*). The rarity of such reports places these in the category of medical curiosities, a designation which also applies to occurrence of the disease in the pigeon (*Columba livia*), goose (*Anser sp.*), canary (*Serinus canarius*), budgerigar (*Melapsittacus undulatus*) (*70*), and mute swan (*Cygnus olor*) (*11*).

The idea that other birds might be susceptible to diseases of the avian leukosis complex occurred to early investigators (*28*). However, experimental attempts to transmit the diseases to pheasants, doves, and guinea fowl (*Numida meleagris*) were unsuccessful.

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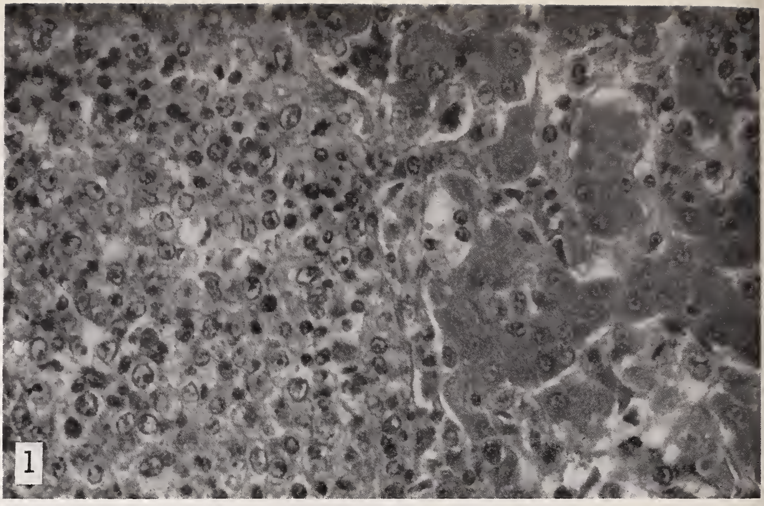

FIGURE 1.—Lymphoid leukosis, liver, hen. Infiltrate is composed of lymphocytic cells. Hematoxylin and eosin. × 480

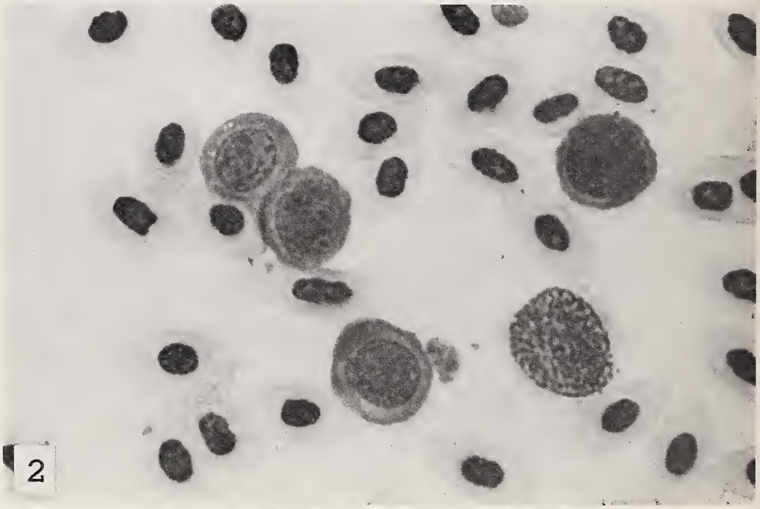

FIGURE 2.—Erythroblastosis, peripheral blood, chick. Field shows typical immature erythroblasts amid normal avian erythrocytes (nucleated). Erythroblastic nuclei tend to be eccentrically placed and chromatin is coarse. Wright-Giemsa. × 1200

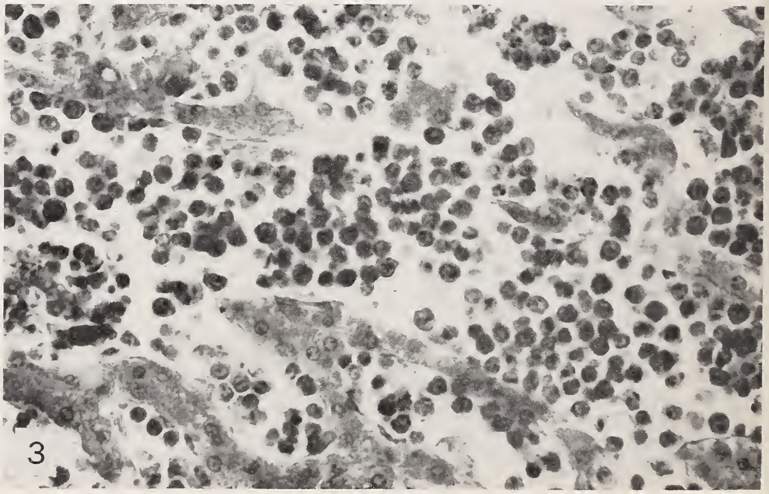

FIGURE 3.—Erythroblastosis, liver, chick. Erythroblasts crowd the sinusoids, a state of "leukostasis." Hematoxylin and eosin. × 480

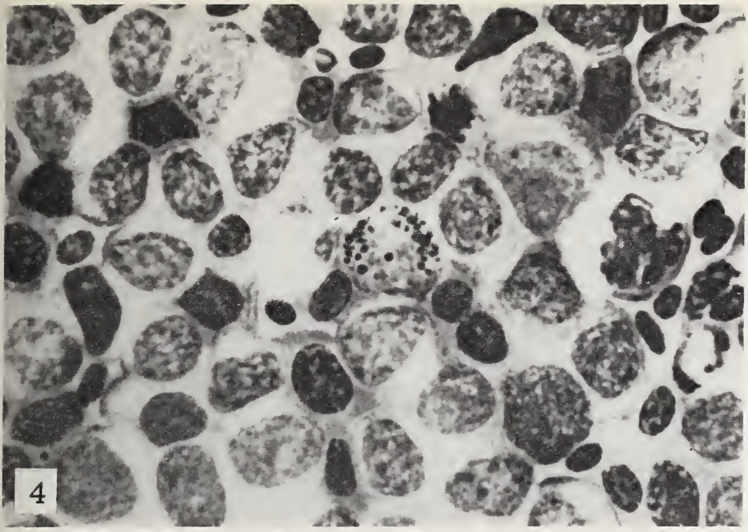

Figure 4.—Myeloblastosis, bone marrow smear, chick. Myeloblasts predominate in field. Nuclear chromatin is finer than that of erythroblasts. Wright-Giemsa. × 1200

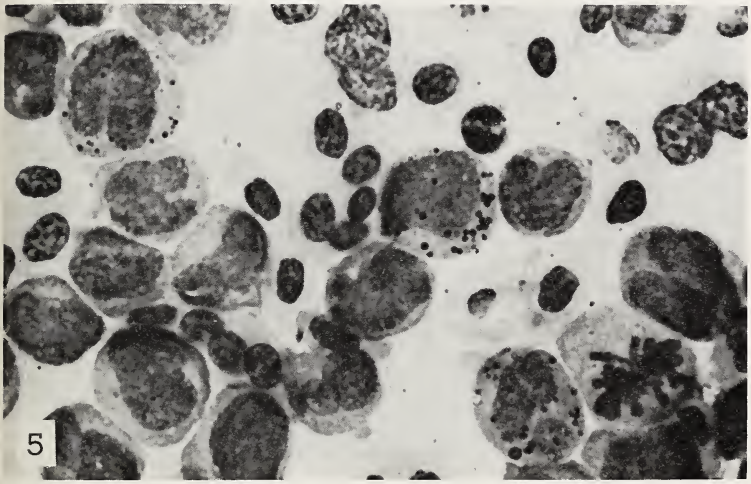

Figure 5.—Myeloblastosis, peripheral blood, chick. Myeloblasts frequently outnumber erythrocytes, and leukocyte counts of more than 1,000,000/mm^3 are not uncommon. *Note* prominent granules in some immature leukocytes. Wright-Giemsa. × 1200

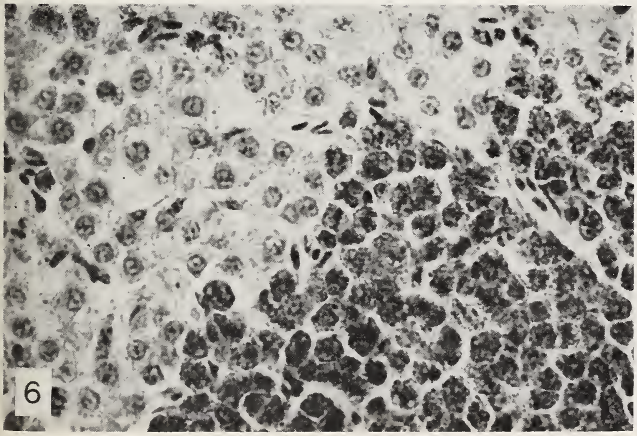

Figure 6.—Myelocytoma, liver, mature hen. Tumor is composed of immature granulocytes. Hematoxylin and eosin. × 480.

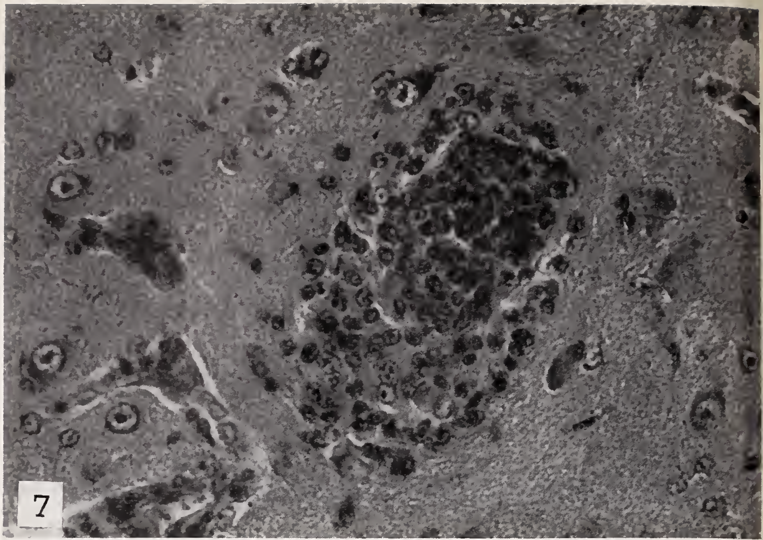

Figure 7.—Marek's disease, brain, chicken. Cells of perivascular infiltrates are lymphocytes. Hematoxylin and eosin. × 4800

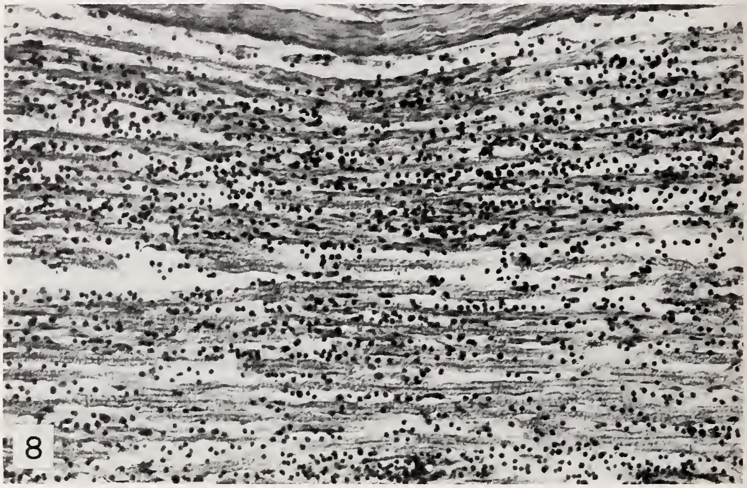

Figure 8.—Marek's disease, peripheral nerve, chicken. Endoneural spaces are infiltrated by lymphoid cells. Hematoxylin and eosin. × 190

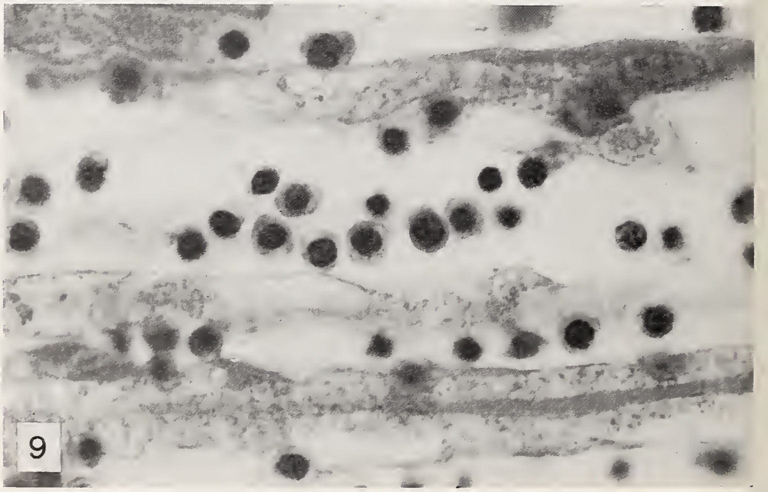

Figure 9.—Marek's disease, nerve, chicken. Higher magnification showing characteristics of infiltrating cells. Hematoxylin and eosin. × 480

Comparative Aspects of Hematopoietic Neoplasms of Rodents [1]

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MOST OF the material I will discuss concerning reticular neoplasms of mice has been published (*1–4*), and I will only emphasize some features of murine diseases of particular interest in comparative hematopathology. I have used the term "reticular" to include both reticuloendothelial and hematopoietic neoplasms, because this term abbreviates a long phrase. The term "leukemia" has been used by some investigators to indicate all reticular and hematopoietic neoplasms of mice, whether or not the process is associated with circulation of neoplastic cells in peripheral blood. The murine neoplasms will be compared to those of other rodents and of man.

Table 1 lists approximate frequencies with which hematopoietic neoplasms are observed in laboratory rodents. Although rabbits are not rodents, it is convenient to include them in discussions of laboratory rodents. In experimental rabbits, hematopoietic neoplasms are observed infrequently, though a recent report from The Jackson Laboratory states that lymphosarcoma, though rare in rabbits, was diagnosed in several strain WH rabbits (*5*). Lopushinsky and Fay reported lymphomas in 6 cottontail rabbits (*6*). In the guinea pig, the lymphocytic type is not uncommon (*7*). In the rat, both lymphocytic and granulocytic neoplasms occur, but the reticulum cell types are relatively infrequent (*8*). In the hamster, reticulum cell neoplasms are known (*9*). In the mastomys, reticulum cell neoplasms are relatively frequent, particularly in old animals (*8*). In the mouse, all types are seen, the frequency and cell types depending on strain and age. The higher frequencies and many cell types observed in mice may be partly explained by the fact that many mice are kept under laboratory conditions and allowed to reach advanced ages. But what is perhaps most important, mice have been inbred and selected for leukemia.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Table 1.—Types of hematopoietic neoplasms in small laboratory animals (Animals are listed in approximate order of frequency with which spontaneous neoplasms are observed.)

Rodent	Type of neoplasm
Rabbit	Lymphocytic
Guinea pig	Lymphocytic
Rat	Lymphocytic, granulocytic
Hamster	Reticulum cell neoplasm
Mastomys	Reticulum cell neoplasm
Mouse	All types (lymphocytic, reticulum cell neoplasms, granulocytic, plasma cell tumors, mast cell tumors)

Table 2 is a rough approximation which attempts to compare reticular tumors of mice with those of man. Stem cell leukemias occur in both species and are quite similar. Lymphocytic neoplasms, which we interpret as a slightly more mature or a more easily recognizable form of monocellular neoplasm, also occur in both species. Good parallels to the granulocytic neoplasms are found in mice, although I seldom see this form because I have not worked with the inbred strains in which these neoplasms are frequent. In the granulocyte of the mouse, the nucleus is usually ring-shaped, and this neoplasm is sometimes difficult to distinguish from a granulocytic leukemoid reaction which is common in the mouse.

Table 2.—Comparison of types of hematopoietic neoplasms of mice with those of man

Neoplasm in mouse	Normal cell	Neoplasm in man
Stem cell	Undifferentiated	Stem cell
Lymphocytic neoplasm, localized	Lymphocyte	Lymphosarcoma
Lymphocytic neoplasm, generalized		Lymphocytic leukemia
Granulocytic neoplasm	Granulocyte	Granulocytic leukemia
Reticulum cell sarcoma, type A	Reticulum cell	Reticulum cell sarcoma
? Hodgkin's-like lesion, type B		Hodgkin's disease
Plasma cell neoplasm	Plasma cell	Multiple myeloma
Mast cell neoplasm	Tissue mast cell	Tissue mast-cell leukemia

Reticulum cell neoplasms are a much more complex group. A well-differentiated, distinctive type of reticular neoplasm occurs in the mouse, which we call reticulum cell, type A. There is no close parallel in man. The reticulum cell, type B, is the closest parallel we know to Hodgkin's disease in man. Type C, which we see infrequently, may be a preneoplastic lesion.

We now have many plasmocytic neoplasms, though, in 1942, Engelbreth-Holm stated that this was one type of neoplasm occurring in man which had not been recognized in the mouse. Since then, we have recognized plasma cell neoplasms developing spontaneously in old mice of inbred strains. These neoplasms appear almost exclusively in the region of the ileocecal valve in C3H mice. They can now be induced easily in BALB/c mice by introducing a variety of substances into the peritoneal cavity. When histologic features of the murine plasma-cell tumors are compared with human multiple myeloma, the plasma cell origin appears to me to be more convincing in the mouse.

Mast cell neoplasms are discussed in a separate paper in this Monograph (*10*).

The lymphocytic forms of reticular neoplasms of mice resemble those observed in man. The lymphocytic neoplasms produced by the Moloney virus resemble the Burkitt tumor and characteristically show the "starry sky" effect associated with phagocytic cells containing chromatin debris.

The reticulum cell neoplasm, type A, which we interpret as a monomorphic tumor, is composed of monocytes and histiocytes and is a fairly well-characterized tumor in the mouse. These cells have a capacity to take on various morphologic forms. The round cells may represent monocytes, and the much more elongated fusiform cells are histiocytic types. One feature of this tumor is that, on transplantation, practically no growth is found at the site of the transplant; instead, a widespread and diffuse growth appears later in the liver and may kill the mouse before there is much other evidence of neoplasia. A peculiar feature of this tumor is the hematoidin crystals occasionally found within the tumor tissue. Since the tumor cells have had no contact with liver cells, they must actually manufacture these crystals, which are a hematoidin-bilirubin type.

The type C reticulum cell neoplasm which we see regularly in one of our high-leukemia strains, the inbred C58, appears to be a true preleukemic lesion. It can be found rarely in isolated lymph nodes of mice of other strains.

The greatest problem is the reticulum cell neoplasm, type B, which we hesitatingly called the Hodgkin's-like lesion in the mouse. This neoplasm certainly resembles Hodgkin's disease of man, in some respects, but we do not insist for a moment that there is a precise identity. The tumor in the mouse is often apparent within the white pulp of the spleen, and involvement in the red pulp is relatively late or absent. The pleomorphic tumor tissue replaces the white pulp and contains a variety of cell forms, but this tissue is well enclosed and bounded by the capsule of the lymph node. This restraint is frequently observed in the type B neoplasm; that is, the tumor rarely spreads beyond the capsule of the node. This neoplasm may involve only a single Peyer's patch of the intestine.

The type B reticular neoplasm also occurs in the wild mouse. Slye stated that she had never seen lymphocytic leukemia in any wild house mouse or in a "tramp mouse." We have always wondered what a "tramp" mouse might be. A colony of wild mice has been kept for a long time at the National Cancer Institute by Dr. Howard Andervont, and in wild mice we occasionally found that the thymus contained the Hodgkin's-like tissue. What we have called "trapped" lymphocytes are within endothelial-lined channels and appear to be normal lymphocytes, held within this tissue. A characteristic feature of this neoplasm in the wild mouse, as in inbred strains, is the cellular pleomorphism. Often cells appear to be plasmocytoid. However, there is usually a background of pale reticulum cells and the cytological details of different cell forms are complex. A considerable collection of lymphocytes may be seen, and in tumors with many lymphocytes it is difficult to decide whether to call the tumor a lymphocytic leukemia or the reticulum cell neoplasm, type B. We do not find any lesions in mice that have all the features of Hodgkin's disease in man, though the neoplasms of the SJL/J strain have some, suggesting a similar process (*11*). However, the murine neoplasm has not shown any cells closely resembling the Reed-Sternberg cell.

I have just published a review paper (*4*) on the reticulum cell neoplasm, type B. I wished before I was half finished that I had never begun, because the whole subject got extremely complicated. Separating this neoplasm from other forms of tumors and coming to some conclusion as to the actual significance of the process became more baffling, the more the problem was explored. However, biological characteristics distinguish the type B reticular neoplasm from others, though one sometimes wonders whether it actually represents a true entity. I believe that it does and that we should attempt to separate it from the other lesions found in mice. The reticular cell neoplasm, type B, occurs only in old mice, and we do not see it with any great frequency except in the SJL/J strain (*11*), though it is fairly frequent in BALB/c mice 2 years of age or older. We have great difficulty in transplanting this tumor, and even after successful transplantation its features may change and it may resemble other types of reticular neoplasms. It has a long and irregular growth period, even after a number of transplant generations. It may take 2–9 months to come up in the first generation, which distinguishes it from the lymphocytic leukemias found in high-leukemia strains of mice which usually transplant easily and grow rapidly. Apparently, a mouse destined to develop lymphocytic leukemia or some form of leukemia at an early age seldom reaches the age at which this reticulum cell neoplasm, type B, might appear. So the two forms of neoplasm are almost mutually exclusive. We have had some experience recently with the Moloney virus that illustrates this point. In untreated BALB/c mice, the virus caused lymphocytic leukemia at an early age, but, if we gave this virus to thymectomized mice, a reticulum cell neoplasm, or a lesion resembling it, often appeared at a much older age. There is a great need for further work on this pleomorphic reticular neoplasm, and the recently introduced NZB and SJL/J mouse strains may be useful for seeking this information. We also need to keep and examine more old mice and perhaps make more attempts at transplantation. Two theories on the genesis of this lesion are now being proposed: 1) It represents or includes an immunological reaction, and 2) it is virus induced. For the present, without more reliable information, we cannot make any final judgment.

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Reticular Neoplasms of SJL/J Mice [1]

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IN 1963, Dr. E. D. Murphy (*1*) of The Jackson Laboratory described a newly developed strain of mouse, SJL/J, which was characterized by a high incidence of spontaneous, pleomorphic reticulum cell sarcomas. The disease was similar to that observed in older mice of other strains (*2*), except that a large percentage of SJL/J mice developed reticular neoplasms before 1 year of age, and incidence approached 100% by 2 years of age. Many other investigators have now confirmed this high incidence of reticular neoplasia with but slight variation (*3–6*). This report describes the reticular neoplasms and characterizes the concomitant immunoglobulin abnormalities.

PATHOLOGY

In mice the disease typically manifests at an average age of 14 months by enlargement of the mesenteric lymph node. Hypertrophy of the cervical, renal, and subcutaneous lymph nodes or spleen also occurs in early stages, or these become involved during progression of the disease. Peyer's patches, liver, and other lymph nodes may also be involved. The thymus is only occasionally involved and the neoplasms are not thymus-dependent. A characteristic feature of the neoplasms is the great increase in the number of reticulum cells in affected lymph nodes and spleen, which results in almost complete replacement of the normal follicular structures (fig. 1). Lymphocytic cells were noted in all tumors, sometimes as collections of "trapped" lymphocytes in the sinuses. Affected lymph nodes had varying numbers of large mononuclear cells with very faintly eosinophilic cytoplasm and large nuclei containing one or more large nucleoli. Nuclear chromatin was aggregated around the nuclear membranes giving an appearance of a halo around the nucleoli (fig. 2). Occasional cells had multiple nuclei. Giant cells of both the Langhan's and the atypical types were seen in most tumors (fig. 3).

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[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Of 50 consecutive reticular neoplasms (table 1), 90% were pleomorphic or of mixed cell types; the infiltrates included histiocytes, plasma cells, lymphocytes, and the giant mononuclear cells. The pleomorphic neoplasms could be further separated into three groups: 1) those in which histiocytes predominate, with few or no plasma cells (22%); 2) those in which plasma cells predominate (48%); 3) mixtures of the two (20%). In 10% of neoplasms, the cell populations were homogeneous and were either lymphocytic or reticulum cell sarcomas, type A (7). The pleomorphic neoplasms with fusiform histiocytes (fig. 4) had a greater degree of diffuse fibrosis than those of any other category except the reticulum cell sarcoma, type A. In contrast, those with rounded or polygonal histiocytes (fig. 5) had very little fibrosis. Neoplasms, predominantly plasmacytic, contained rounded cells with large basophilic cytoplasm (fig. 6). Nuclei had dense chromatin patterns and were usually eccentrically located (fig. 7). Juxtanuclear clear zones were observed in many of these cells. The cells appeared to be mature and many showed Russell body morphology (fig. 8). The mixed tumors were usually found within a single node, but in some mice, histiocytic patterns were observed in one node and a plasmacytic neoplasm in another.

The monomorphic reticular neoplasms of SJL/J mice were reticulum cell sarcomas, type A (4%), and lymphocytic neoplasms (6%). Both types were transplantable and continued to grow as a single homogeneous cell type.

Of 16 primary neoplasms transplanted subcutaneously, 12 grew progressively in syngeneic recipients as a generalized process involving the lymph nodes, spleen, and liver. The pleomorphic tumors grew as mixed cell types through several transplant generations, but later became primarily reticulum cell tumors of the histiocytic type or failed to grow.

IMMUNOGLOBULINS

Because many neoplasms of SJL/J mice were composed of plasma cells, we examined the serum proteins by agar gel electrophoresis. One-third to one-half of mice with definite neoplasms had elevated immunoglobulins (fig. 9). The abnormal sera were typed by immunoelectrophoresis using appropriate rabbit antisera made against mouse myeloma proteins (*8*). Of the first 20 sera, 12 had a fast γ or γF elevation; 4 had slow γ of γH type, and 1 had a slow γ of γG type (fig. 10). Three tumors produced two different immunoglobulins. None had elevated γM or γA nor had Bence Jones proteinuria. Eleven immunoglobulin-producing tumors grew in transplant and 5 of these produced the same type of protein as the primary tumor. Five did not produce elevated immunoglobulin in transplant and one apparently produced a protein of a different type in transplant. The

TABLE 1.—Classification of 50 consecutive reticular neoplasms in SJL/J mice

Neoplasms	Number	Percent
Pleomorphic		
1) Histiocyte-predominant		
a) Round	3	6
b) Fusiform	8	16
2) Plasma cell-predominant	24	48
3) Mixed—histiocyte and plasma cell	10	20
Monomorphic		
1) Reticulum cell sarcoma, type A	2	4
2) Lymphocytic neoplasm	3	6

ability of the tumor in transplant to produce immunoglobulin is usually lost in the second to the sixth generation, sometimes, but not always, coincident to loss of the tumor line.

DISCUSSION

The exact nature of the SJL neoplasm is not known. We do not know which cells are neoplastic or whether the neoplasm is monofocal or multifocal in origin. In support of a monofocal origin are the following: 1) Most tumor-associated, increased immunoglobulins were of a single type; 2) the primary tumor could have spread from a single focus to involve many nodes and spleen, just as transplanted tumors spread from the site of inoculation and grow in multiple distant sites; and 3) the pleomorphic histology could be due to differentiation of a multipotential cell, and the observed loss of protein secretion by tumors during the transplantation could represent a reversal of dedifferentiation of the protein-synthesizing cells. A multifocal origin is supported by: 1) the heterogeneity of cell type within the pleomorphic neoplasms and wide spectrum of disease with apparent overlapping and mixtures which occur; 2) the stability of many heterogeneous tumors which remain heterogeneous in transplant; and 3) the ability of mice with some tumors to produce more than one paraprotein. Loss of paraproteins during transplantation could be explained as a preferential growth of non-protein-producing cells.

Future studies of SJL neoplasms will be anticipated to provide additional information about their pathogenesis and may also provide a better understanding of the potentiality of cell differentiation within the immune system.

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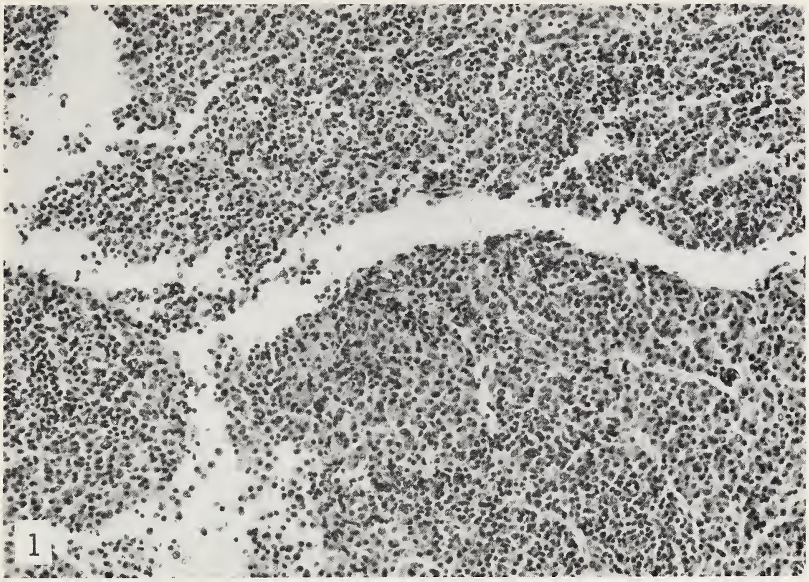
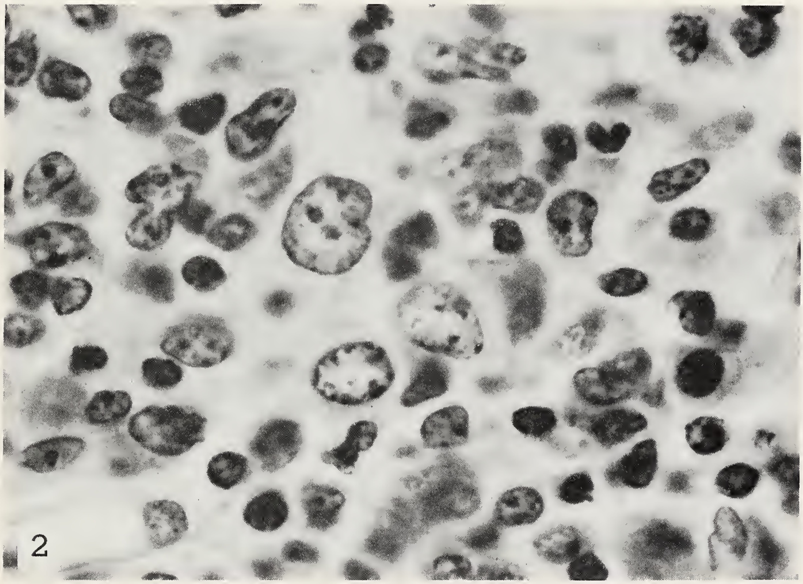




All cells stained with hematoxylin and eosin

Figure 1.—Lymph node from primary tumor K-5772. Mesenteric node filled with histiocytes, reticulum cells, plasma cells, and lymphocytes, with replacement of normal follicular structure. × 160

Figure 2.—Lymph node from primary tumor J-96934. Large mononuclear cells with prominent nucleoli and chromatin material aggregated around nuclear membrane. × 1250

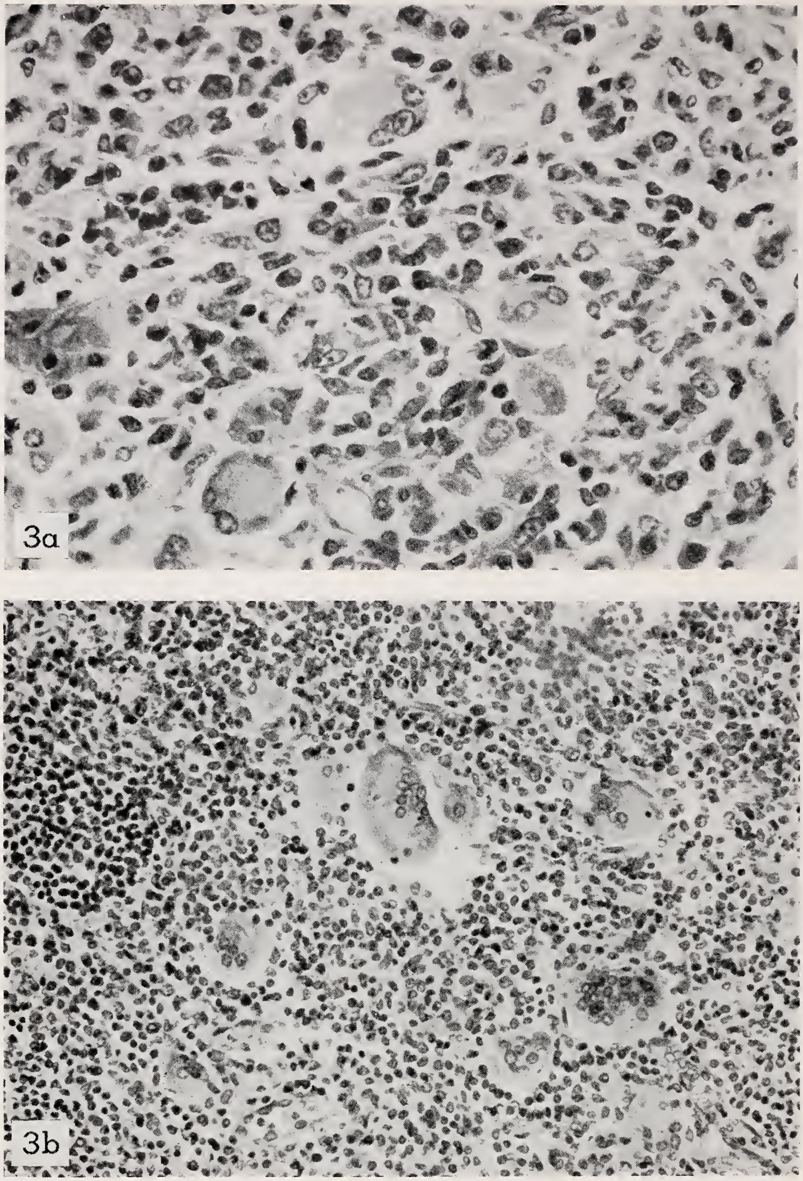
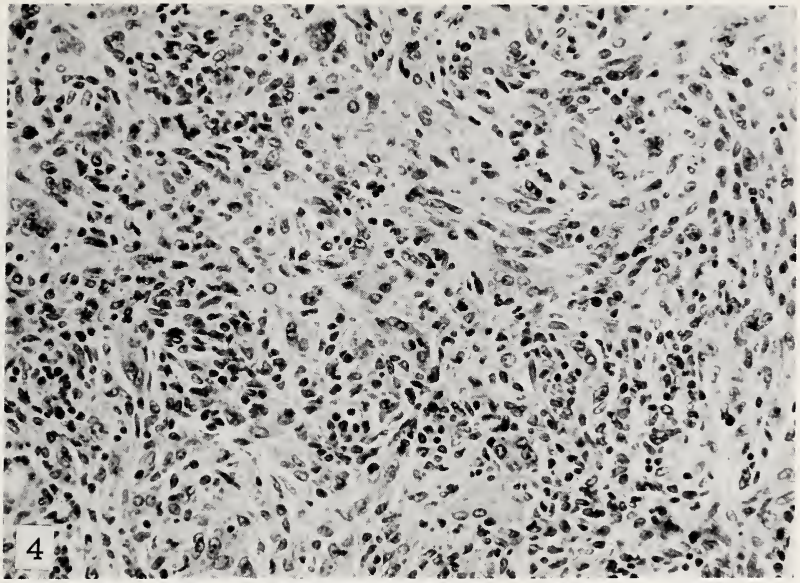


FIGURE 3.—(*a*) Lymph node from generation 1 of tumor K-5796. Several Langhan's type giant cells and many histiocytes, reticulum cells, and infiltrating lymphocytes are evident. × 500

(*b*) Thymus from primary tumor J-96934. Several large atypical giant cells are seen. × 260



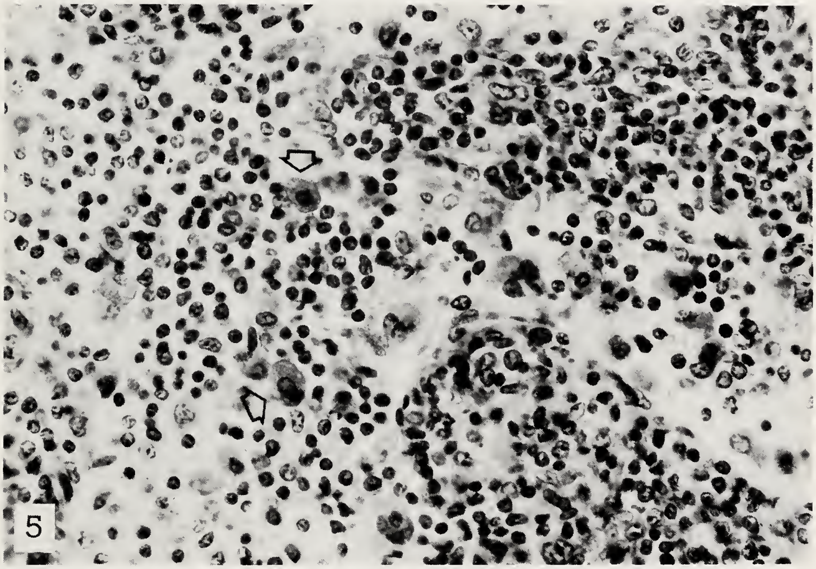
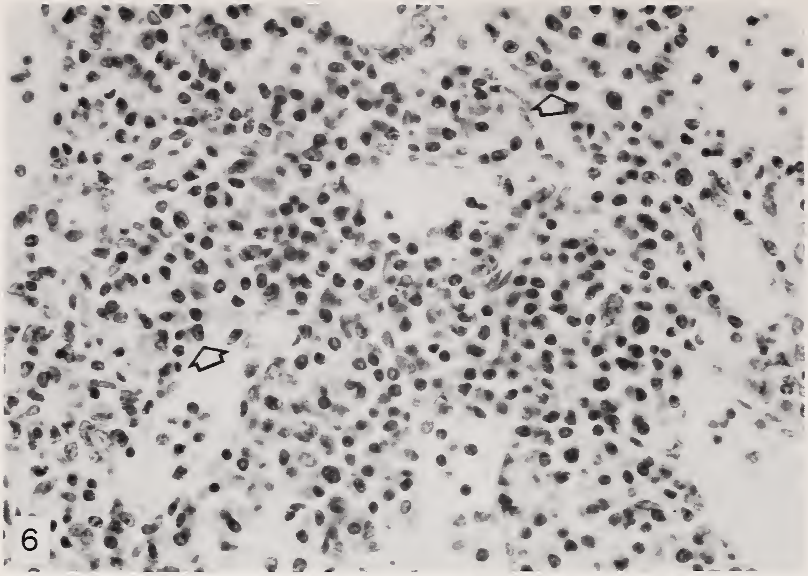


Figure 4.—Lymph node from primary tumor J-96936. Predominant cell type is a fusiform histiocyte in the midst of moderate fibrosis. There are also infiltrating lymphocytes. × 260

Figure 5.—Lymph node of primary tumor K-581. Normal follicular structure replaced by rounded histiocytes. *Note* tissue mast cells (*arrows*). × 400



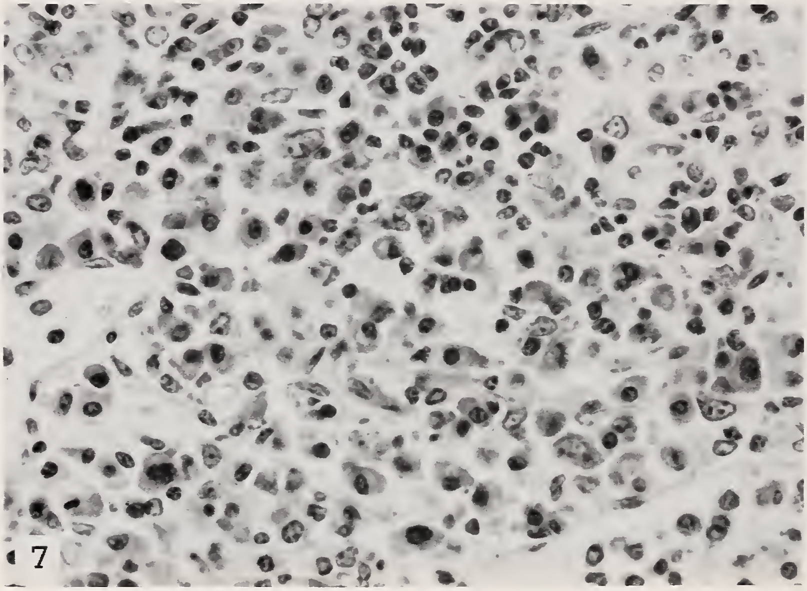
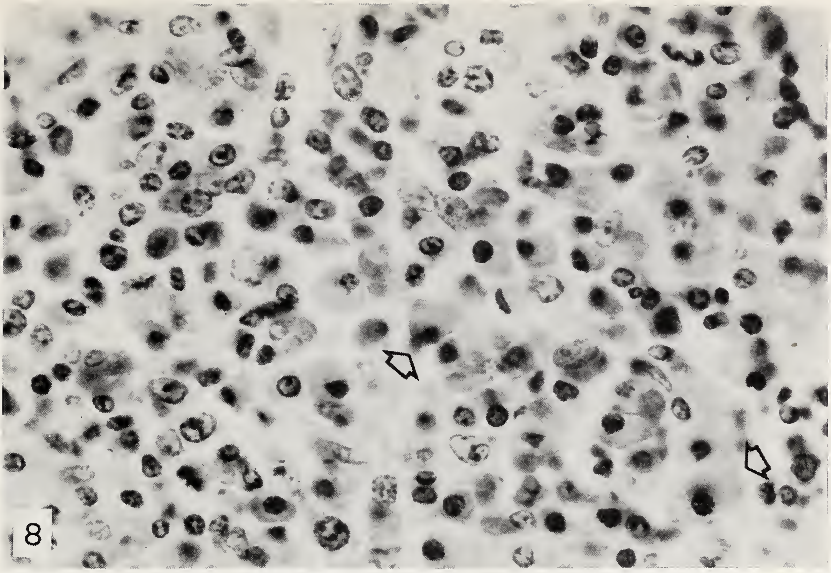


FIGURE 6.—Lymph node of primary tumor J-96934. Typical plasma cell hyperplasia showing mature cells, many with juxtanuclear clear zones (*arrows*). × 400

FIGURE 7.—Lymph node from generation 1 of tumor K-5796. Large mature plasma cells with prominent juxtanuclear clear areas and many reticulum cells, histiocytes, and lymphocytes. × 500



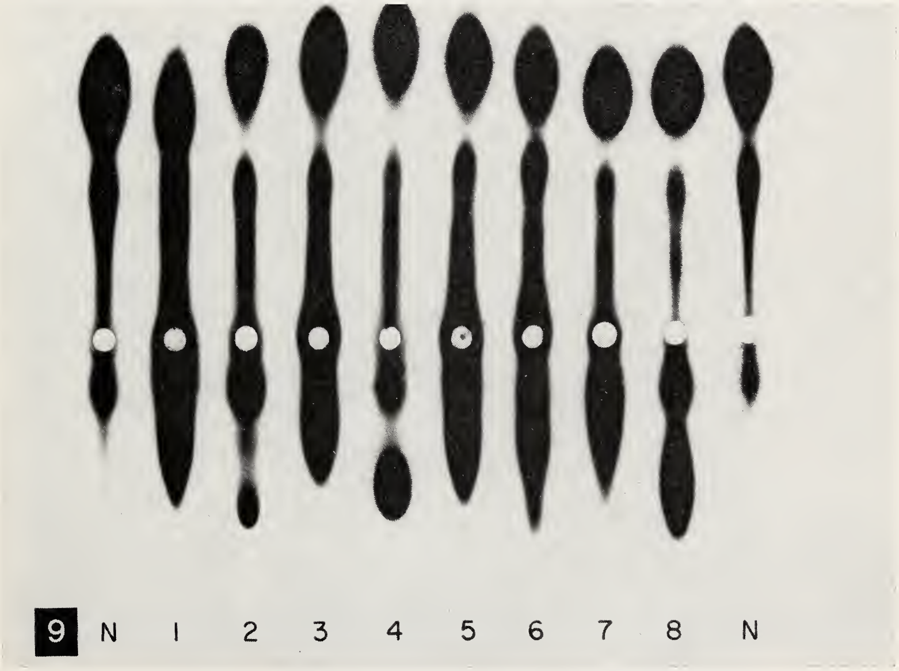


Figure 8.—Lymph node of primary tumor K-2106. Plasma cell hyperplasia with many Russell body forms (*arrows*), reticulum cells, and lymphocytes. × 630

Figure 9.—Agar gel electrophoresis of normal SJL/J serum (N) and sera from 8 mice with different tumors. The anode is toward the *top* and the abnormal immunoglobulins migrate between the origin wells and the cathode at the *bottom*. Sample 4 represents the first transplant generation of tumor K-5796; the rest are from primary tumors.

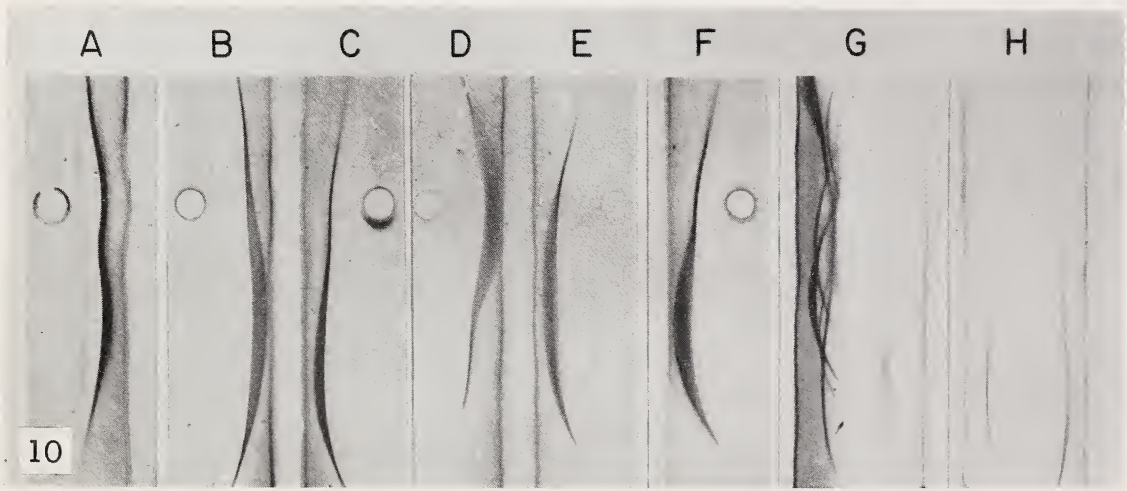


Figure 10.—Typing of abnormal immunoglobulins using immunoelectrophoresis and specific antiserums. Cathode is toward the *bottom*. A is reacting with anti-γH, while B through F is reacting with γF. (*Note* the variety of mobilities possible within this single immunoglobulin type.) G and H are normal SJL/J serum reacting with (from *left* to *right*) anti-whole serum, anti-γH, anti-γF, and anti-γG.

Hematopoietic Neoplasms of Rats and Mastomys [1]

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IT IS sometimes difficult for those of us who work with rats and mice and Mastomys and men to agree upon a diagnosis for hematopoietic neoplasms, because of our lack of agreement on the definition of such terms as "leukemia," "lymphocytic neoplasm," "malignant tumor of the lymphoreticular system," or "malignant lymphoma." We often have a certain tenacity for our own favorite terminology and do not define explicitly our terms. The situation reminds me of a quotation from *Alice Through the Looking Glass* when Humpty Dumpty said, "When I use a word, it means exactly what I choose it to mean, neither more nor less." And Alice said, "The question is, whether you *can* make a word mean so many different things?"

Although all of us admit that even after careful study there are a few hematopoietic neoplasms that cannot be definitely categorized, most of those that occur in animals at least, and have no malignant white cells in the blood, can be diagnosed as lymphosarcoma, reticulum cell sarcoma, or plasma cell myeloma. Those that do have malignant white cells in the blood can usually be diagnosed as granulocytic or lymphocytic leukemia.

In enumerating tumors of rats, various investigators have diagnosed lymphosarcoma as occurring in the mesenteric or cecal lymph node, in the thymus, in the mediastinal lymphoid tissue, and in the lung (*1-5*). The number and types of these neoplasms vary greatly from laboratory to laboratory and even among rats of the same strain. Futhermore, the differential diagnosis between lymphosarcoma and cecitis and lymphosarcoma and the common peribronchial inflammation of the rat can be so difficult that it is really uncertain how many times this neoplasm occurs. In particular, the English workers have compared the lungs of specific pathogen-free rats with those of conventional or "dirty" rats. They suggested that, since lymphosarcomas of the lung were confined to the "dirty" rats, the neoplastic process is related to the enormous accumulations of lymphocytes that are a feature of chronic murine pneumonia. They further suggested that the chronic inflammation may play a part in the evolution of lymphosarcoma. So far as I know, definitive experiments to prove or disprove this theory have yet to be carried out.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

In the course of studying several hundred rats from the various inbred strains maintained at the National Institutes of Health (NIH) (*6*), we have encountered two lymphosarcomas confined to the lung. Both lymphosarcomas were in Buffalo (BUF) strain rats. The neoplastic cells were congregated particularly around blood vessels and sometimes around bronci and bronchioles. The chief cell was a large atypical lymphocyte with a hyperchromatic, often indented nucleus; the usual array of normal-appearing lymphocytes and other inflammatory cells was not present. We have an example of a lymphosarcoma confined to the cecum and the bone marrow of a BUF rat and another example of one in a BUF rat that involved nearly every organ of the body (lungs, liver, spleen, lymph nodes, bone marrow, kidneys, heart, pancreas, sex organs, alimentary tract, and brain). Both neoplasms had a rather monotonous pattern of neoplastic lymphocytes with a few vacuolated histiocytes.

Reticulum cell sarcoma is so rare in NIH inbred rats that I have but one case, and this was confined to the colon and a regional lymph node of an M520 rat. The predominant reticulum cell had eosinophilic cytoplasm and a heavily stained basophilic nucleus that varied greatly in shape and size. In some sections there were spindle cells and multinucleated giant cells as well. In Wilder's silver preparations, reticulum fibers were around small groups of cells.

We have diagnosed leukemia in one rat in each of the inbred Fischer (F344), BUF, and ACI strains, but have found no leukemia in the Osborne-Mendel (OM) or M520 rats. About 14% of our Wistar rats over 18 months old have granulocytic leukemia. The greater tendency for Wistar rats to develop leukemia was also noted by Kim, Clifton, and Furth (*7*) who reported leukemia in 6 of 83 of an inbred Wistar line similar to ours.

To our knowledge, spontaneous leukemia in the rat was not reported until 1936, when Wilens and Sproul (*8*) described 12 cases in OM rats over 20 months old. All but one of these neoplasms were granulocytic. Other cases of spontaneous leukemia in rats have been reported (*9–15*), and Engelbreth-Holm (*16*) reviewed extensively leukemia in animals.

The Mastomys, a South African rodent, is coming into use as a laboratory animal. The scientific name is *Praomys* (*Mastomys*) *natalensis* (*17*). The *Mastomys*, a distinct subgenus of rodent, is intermediate in size between the rat and the mouse. These animals have a pattern of disease different from other rodents: Mastomys spontaneously develop malignant argyrophilic carcinoids of the glandular stomach (*18*), a severe form of degenerative joint disease (*19*), glomerulonephritis similar to the type seen in man (*20*), thymomas, and thymic hyperplasia, which is sometimes accompanied by myositis, myocarditis, and various inflammatory cell infiltrates in different organs that suggest the possibility of a disease akin to myasthenia gravis (*21, 22*). They also develop a variety of hematopoietic neoplasms (*23*) which we have categorized as lymphosarcoma, reticulum cell sarcoma, and plasma cell myeloma. These neoplasms appear to be diseases of old age, since only 2 occurred in animals less than 2 years old and the rest occurred in animals whose ages were evenly distributed between 26 and 38 months. Nearly all Mastomys of our necropsy series of 213 animals were allowed to live as long as possible, so that our data derive almost entirely from old animals. None of the animals had leukemia blood. There were 5 lymphosarcomas, 3 of which had a nodular, follicular pattern. The nodular, follicular lymphosarcomas involved the spleen, all sets of regional lymph nodes, the bone marrow, and the lymph nodules of the intestine. The diffuse lymphosarcoma had a wide visceral distribution in one animal, but was confined to the lymph nodules and wall of the intestine and a regional lymph node in the other. All 5 lymphosarcomas were composed of hyperchromatic, neoplastic, variably sized lymphocytes with little cytoplasm. The nucleus was 10–15 μ in diameter; it varied somewhat in shape but little in size, and it had many mitotic figures.

Reticulum cell sarcoma was diagnosed in 12 animals, 2 males and 10 females. Of the neoplasms, 7 were classified as well differentiated, 3 as anaplastic, and 2 as a mixture of these with the addition of areas that resembled lymphosarcoma. The degree of differentiation of the neoplasm and the sites of involvement were correlated. The well-differentiated reticulum cell sarcomas primarily involved the spleen, lymph nodes, intestinal lymph nodules, and bone marrow, with pancreatic and mesenteric deposits in 3 animals representing extensions from abdominal lymph nodes. By contrast, the anaplastic reticulum cell sarcomas had a wide visceral distribution. The extent of involvement of the spleen by the different types of reticulum cell sarcoma varied from grossly imperceptible deposits to massive gray or tan nodules. The weight of the spleen generally reflected the degree of neoplastic involvement, but excessive hematopoiesis and follicular hyperplasia, caused by extrasplenic hemorrhage, infection, or thrombosis in a few cases, contributed more than the neoplastic tissue to the splenic weight. Of the lymph nodes, the cecal was the most consistently enlarged; in one animal it measured 2.5 cm in length. But the pancreatic, gastric, and retroperitoneal lymph nodes were also conspicuously enlarged in some animals. The livers from the animals with anaplastic and mixed neoplasms were enlarged, but none to more than twice their normal size. Like the spleen, these livers contained gray or tan nodules. Gross deposits of tumor tissue at other sites, such as kidneys, ovary, and peritoneum, were also noted at necropsy, although some deposits were detected only upon histologic examination.

If one considers the wide limits Dunn (*24*) has established for reticulum cell neoplasm Type B of mice, probably the entire group of reticulum cell sarcomas in Mastomys can be assigned to this category. The 7 well-differentiated reticulum cell sarcomas were composed of neoplastic cells that were fairly uniform in size, shape, and staining. The cells had a small amount of pale cytoplasm, a pale, open, oval nucleus, often with a single nucleolus, and a small amount of chromatin. A few cells were larger and had a bizarre, single nucleus or multiple nuclei. Inconstantly intermingled with the neoplastic reticulum cells at some sites were small, atypical lymphocytes that constituted a minor part of the neoplasm. In the 3 animals with anaplastic reticulum cell sarcoma, the cells were pleomorphic and intermingled with the tiny atypical lymphocyte and a few plasma cells. The cytoplasm was abundant, often amphophilic, and sometimes contained granular, eosinophilic, periodic acid-Schiff-positive material. Large amounts of reticulum surrounded single cells in all animals, and mitotic figures were numerous and bizarre.

The one Mastomys in which plasma cell myeloma was diagnosed was killed because of its poor physical condition and unsteady gait. At necropsy a large, pale, nodular, firm, and homogeneous tumor was found to lie posterior to the esophagus, trachea, and thyroid gland. It had destroyed the anterior and right lateral portions of a cervical vertebra and extended into the spinal canal, where it compressed the cord and invaded a nerve and ganglion. This probably accounted for the signs noted before death. The tumor was composed of loosely arranged sheets of neoplastic plasma cells with eccentric cartwheel nuclei, abundant cytoplasm, and a perinuclear hof. The cells varied moderately in size, and some were multinucleated. The plasma cell myeloma readily transplanted and is now in its 35th generation.

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Morphology of Cavian Leukemia [1,2]

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HISTORICALLY, cavian leukemia appears to be a relatively rare disease. Sporadic cases of what appeared to be leukemia in guinea pigs were seen by various individuals as early as 1918, with isolated examples of leukemia occasionally being documented in various parts of the world. Heston and Deringer, in 1952, described 2 cases in 51 strain-2 guinea pigs which had been treated with intravenous injections of dibenz[*a,h*]anthracene and 3-methylcholanthrene (*1*). In 1954, a series of 10 cases of guinea pig leukemia was reported, with incidences of 3.6% occurring in 112 strain-2 guinea pigs, 6.7% in 30 strain-13 animals, and 2.5% in 161 heterogeneous hybrids (*2*). The true incidence of leukemia is obscure because of lack of complete autopsy and hematologic studies in those animals dying suddenly of acute disorders. Due to the paucity of findings in acute cases compared to findings in more slowly developing dyscrasias in which clinical signs of disease are observed, significant features of leukemia could be easily overlooked in a cursory examination. The time-honored custom of many large-scale breeders of guinea pigs of destroying animals with spontaneous diseases without adequate pathologic examinations has contributed to the uncertainty of the number of cases of leukemia. Additionally, many investigators using guinea pigs experimentally in the past were more concerned with deficiency, bacterial, and hypersensitivity disorders than with neoplastic diseases. By adopting the policy of carefully examining the bone marrow and peripheral blood, as well as necropsy examination of all dead animals, it has been determined that the spontaneous occurrence of leukemia in our outbred and inbred guinea pig colonies is approximately 2.5% in more than 2,000 animals studied. Cases of spontaneous leukemia in stain-2, Hartley, and outbred animals have been documented. Radiation apparently does not increase the incidence of leukemia in the animal colonies we have studied. Guinea pigs have been inbred since 1897, and studies on these original families have been published (*3*). With regard to

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age incidence, the disease appears to occur in animals over 1 year of age, while other neoplasms occur in guinea pigs over 3 years of age.

Leukemia research has mainly made use of the widely known avian and murine systems. With isolation of the Opler virus (OV) as a casual agent for cavian leukemia (*4–7*) and the proved value of guinea pigs in comparative pathology of other diseases, this system may have potential value as an experimental model for leukemia.

In our laboratory, cell-free material containing the OV in high-speed plasma pellets routinely produced the disease after injection into strain-2 guinea pigs or F_1 hybrids, as well as resulting in occasional successful takes in outbred guinea pigs. We have also found it possible to transmit the disease by the oral feeding of leukemic spleen cells (*8*). The virus has been regularly recovered from the plasma of strain-2 guinea pigs with leukemia and has not been observed in the absence of the disease. The types of leukemia seen are almost universally lymphatic, some appearing to be reticulum cell sarcoma leukemia or stem cell leukemia. Cavian leukemia is much like acute lymphatic leukemia in man (*9*).

The animals in this experimental model presented the classical picture of an acute leukemia running a rapid clinical course, with terminal cell counts ranging from 125,000–300,000 blast cells/mm^3 in the peripheral blood. A detailed pathologic review of this disease derived from the study of animals in our colonies has been previously reported (*10*). Briefly, all organ systems studied, including heart, lungs, and genitourinary tract, were strikingly infiltrated with leukemic cells. Leukemic involvement of the retina and pia arachnoid of the meninges was routinely observed in advanced cases. The lymphoid tissue throughout the body was massively involved.

CYTOMORPHOLOGY

Gross examination of guinea pigs with spontaneous leukemia revealed that the bone marrow was pink-gray and markedly cellular. Under the light microscope, the marrow spaces were seen to be filled with leukemic cells which replaced marrow elements; only rare megakaryocytes, granulocytic cells, and normoblasts persisted (fig. 1). Occasional areas of uninvolved fat, diminished in amount, were seen in early cases. High-power magnification revealed undifferentiated cells with coarse nuclear borders, and sometimes lobulated vesicular nuclei appeared in large sheets or islands (fig. 2). The leukemic cells measured 10–20 $m\mu$ in diameter. A few cells had one or several nucleoli which stained deep blue with Giemsa and purple with hematoxylin and eosin. Numerous mitotic figures, including occasional abnormal configurations, were present. Most cells in the peripheral blood appeared identical to those in the marrow (fig. 3). No Auer rods were present. There was a pronounced decrease or absence of granulocytes. No peroxidase-positive or neutral red granules were seen in the neoplastic cells. Foa-Kurloff bodies (*11, 12*), arising in the lymphocytes, were observed. They contained large homogeneous inclusion bodies with relatively flat nuclei pressed against their cell borders. These cells appear in healthy guinea pigs and are not observed to increase in the course of the leukemia. Electron microscopy of these cells at different stages of development revealed that these bodies were in the cytoplasm, had smooth surfaces, and were rather homogeneous and osmiophilic, regardless of size. The proliferating leukemia cells were stained with uranyl acetate and on electron microscopic study showed dense staining and condensation of chromatin at the periphery of the nucleus. These cells had the characteristics of lymphoblasts (fig. 4). In the parachromatin area of these cells, wide bands or islands were clearly visible in the light background. The usual lymphoid cell had one or two nucleoli which appeared to be round and less dense than the chromatin. The cytoplasm was moderately dark, and abundant ribonuclear protein particles were present. The mitochondria were round or rod-shaped and distributed throughout the cytoplasm. The Golgi apparatus was small, with granules and vacuoles present. The inner and outer nuclear envelope produced a perinuclear light space due to separation. Viral particles budded into the cisternae of the endoplasmic reticulum. In its immature form, this virus had an intermediate layer that was less electron-dense than those of the murine leukemia viruses. Numerous mature type C particles were seen in intracellular spaces. No virus particles were observed budding from the cell membrane, but they were readily observed in the cytoplasm of these infiltrating cells.

The thymus was slightly enlarged, but did not form the conspicuous mass observed in other animal leukemias. Hassall's corpuscles were not obliterated, but the parenchyma contained numerous lymphoblasts. The architecture was partially preserved and the leukemic cells had the morphology of those seen in the peripheral blood and bone marrow (fig. 5). The lymph nodes were markedly enlarged and strikingly visible on gross examination. The architecture of the nodes was obliterated and on microscopic examination there was invasion of the capsules. Under high-power magnification, the nodes were seen to have been extensively replaced with lymphoblasts (fig. 6). The liver was enlarged up to two and a half times normal size. The spleen was six to ten times normal size. On microscopic examination, it was observed that the periportal spaces of the liver were infiltrated with leukemic cells having the morphology of lymphoblasts (fig. 7).

The low incidence of acute leukemia in guinea pigs is comparable to that found in humans. Comparative morphology of acute leukemia in guinea pigs and humans reveals many similarities.

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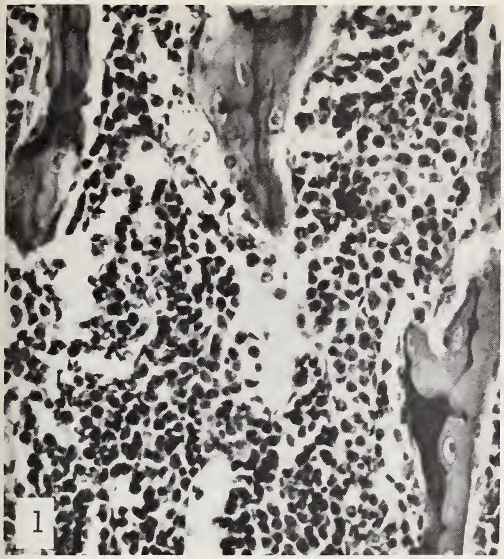
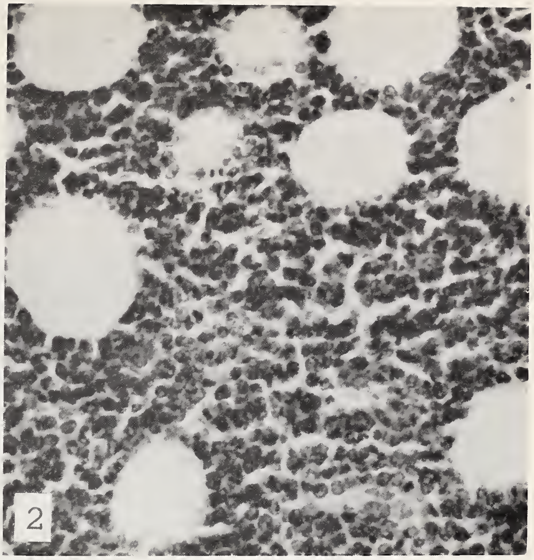




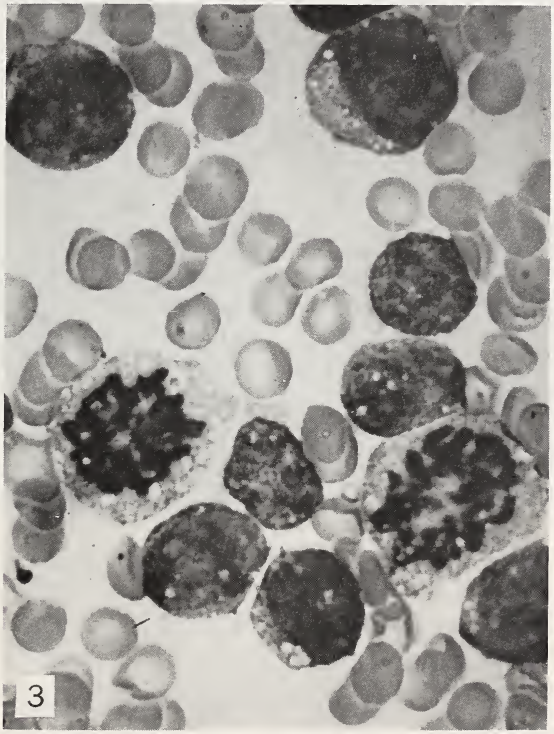


Figure 1.—Bone marrow spaces with leukemic cells replacing the marrow elements. Approximately × 150

Figure 2.—Bone marrow; high-power view of lymphoblasts. Approximately × 600

Figure 3.—Peripheral blood; mitotic figures and lymphoblast, showing nucleoli. Approximately × 2400

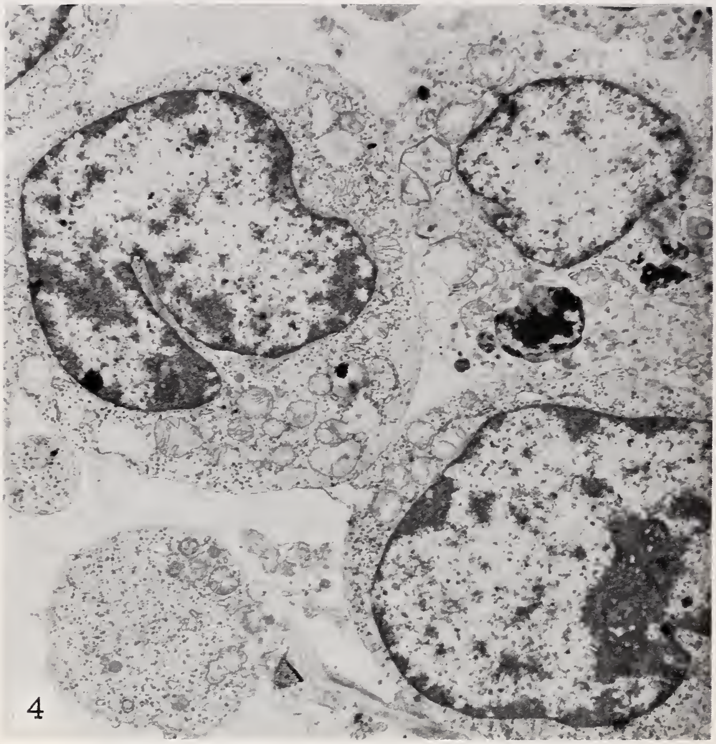
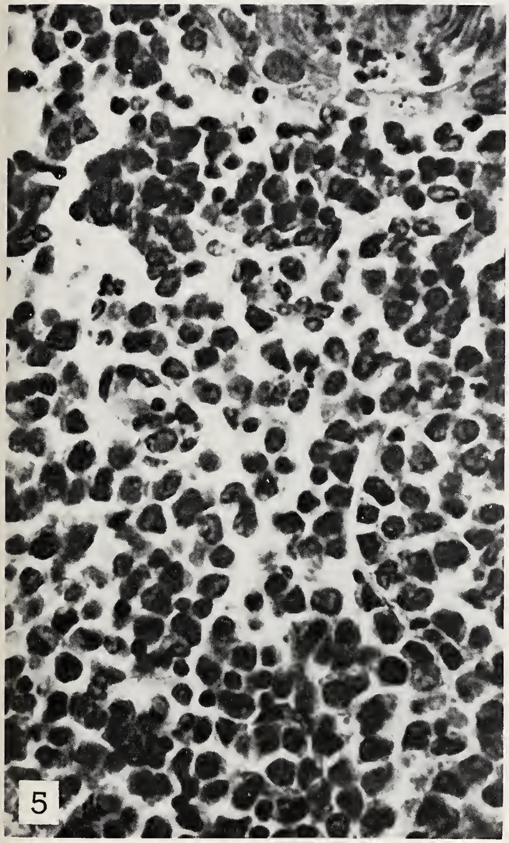


FIGURE 4.—Bone marrow; electron microscopic view of lymphoblasts. Approximately × 9000



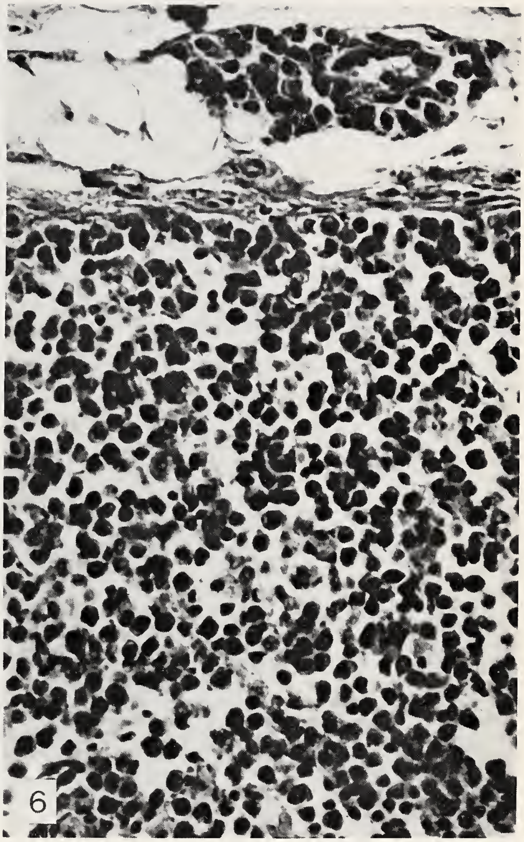


Figure 5.—Thymus; lymphoblasts infiltrating thymic tissue, with portion of Hassall's corpuscle seen. Approximately × 240

Figure 6.—Lymph node; replacement of node with lymphoblasts and invasion of capsule. Approximately × 150

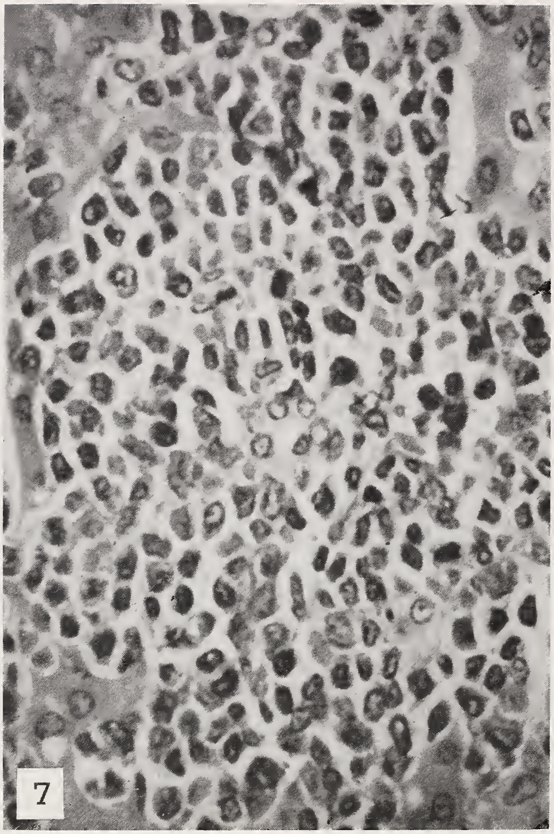


FIGURE 7.—Liver; periportal spaces infiltrated with lymphoblasts. Approximately × 240

Spontaneous Hematopoietic Neoplasms of the Domestic Cat [1, 2]

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HEMATOPOIETIC NEOPLASMS are the most prevalent neoplasms of the domestic cat and account for almost one-third of all feline neoplasms. Of domestic animals, the high incidence of spontaneous hematopoietic neoplasms observed in cats is surpassed only by that in mice of inbred strains specifically bred for susceptibility to leukemic neoplasms as AK albinos and C58 blacks, and by that in chickens of certain flocks exposed to leukemogenic viruses.

Hematopoietic neoplasms of cats can be classified according to the scheme outlined in table 1, which is based on cytomorphology of malignant cells and distribution of infiltrates. Accurate and consistent cytomorphologic diagnoses require hematologic information and an opportunity to examine well-stained imprints of nodes or other organs and smears of peripheral blood and bone marrow. Histologic material fixed in a variety of solutions, prepared by different techniques, and cut in varying thicknesses considerably distorts cells, which results in variations of interpretations by different investigators. Only adoption of uniform methods of preparation of material and criteria for identification of cells will allow accurate comparisons to be made between hematopoietic neoplasms of animals studied in different laboratories.

LYMPHOSARCOMAS

Lymphosarcomas are initially localized lymphoid tumors accounting for approximately 90% of all feline hematopoietic neoplasms and are the most frequently observed of all malignant neoplasms of cats. The term "lymphosarcoma" is considered to be synonymous with "malignant lymphoma" (*44*) and is preferred by many investigators, because it best

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Table 1.—Classification of feline hematopoietic neoplasms

Lymphosarcoma	Stem cell	
	Pleomorphic	
	Lymphoblastic	
	Lymphocytic	
Lymphoid leukemia	Lymphocytic	
	Lymphoblastic	
Reticulosis (reticuloendotheliosis)		
Mastocytosis	Localized (skin)	
	Systemic or diffuse	
Myeloproliferative	Granulocytic	Eosinophilic
		Neutrophilic
		Basophilic
	Erythrocytic	
	Monocytic	
	Plasmocytic	

describes the "solid" or "sarcomatous" character of the neoplasms observed in cats and clearly differentiates it from diffuse or leukemic lymphoid neoplasms. The term "visceral lymphosarcoma" used by some authors refers to the tendency of the feline tumor to occur in single or multiple masses involving internal viscera and lymph nodes and to remain localized in these sites for relatively long periods. In contrast to classic lymphoid neoplasms of cattle and dogs, in which early involvement of superficial lymph nodes is characteristic, generalized superficial lymph node enlargement is rare in cats.

In 1953, Nielsen and Holzworth (*38*) reviewed the literature describing feline lymphosarcoma. This review and a study of 100 additional cases from the Department of Animal Diseases of the University of Connecticut provided material for the following characterization of feline lymphosarcoma.

Histopathology

At least 4 cell types of feline lymphosarcoma can be recognized, each representing a stage of differentiation from the stem cell to the mature lymphocyte (table 1).

1) *Stem cell.*—This lymphosarcoma is a monotonous and closely packed population of the largest and least differentiated cells in the lymphoid series (figs. 1 and 2). Typically, the cells have ovoid or round vesicular nuclei, thin nuclear membranes, and single, small nucleoli. Mitoses are frequent. Cytoplasm is pale with indefinite borders. In approximately one-half of the cases, numerous large clear histiocytes give a "starry sky" appearance. The feline stem cell lymphosarcoma most nearly resembles the human *undifferentiated* or *stem cell* malignant lymphoma (*44*).

2) *Pleomorphic.*—This lymphosarcoma is a heterogenous population of cells, most of which are neoplastic histiocytes. Nuclei are vesicular and indented. Wide rims of pale cytoplasm have fairly well-defined borders (fig. 3). Scattered throughout are various mature lymphocytes

and/or mononuclear giant cells 2–4 times the size of the histiocytes. Binucleate giant cells, similar to Reed-Sternberg cells of human Hodgkin's disease, are occasionally observed. Of feline lymphosarcomas, the pleomorphic form is observed much less frequently than other cell types; only 2 were included in the 100 cases of the Connecticut series. The variability of component cells, many of which are clearly inflammatory, as have been described in pleomorphic reticular neoplasms of mice and other animals, has resulted in difficulties in precise classification and comparisons between species. The feline neoplasm resembles *pleomorphic histiocytic* neoplasms of man. In one study, Weiss (*59*) used the German term "Retothelsarkom" to designate a feline tumor consistent with the pleomorphic lymphosarcoma described in this study.

3) *Lymphoblastic.*—This lymphosarcoma is a relatively uniform population of immature lymphocytic cells. The nuclei are round or slightly ovoid and have heavy chromatin and single large prominent nucleoli (figs. 4–6). Mitoses are frequent. The cytoplasm stains well, forms a wide rim, and has a distinct border. Scattered, clear histiocytes are frequent in some. Impression smears stained with Giemsa reveal lymphoblasts which measure 12–14 μ and have bluish cytoplasm, dark nuclei, and distinct nucleoli. The lymphoblastic type of feline lymphosarcoma corresponds most nearly to the human *poorly differentiated lymphocytic lymphoma* (*44*).

4) *Lymphocytic.*—This lymphosarcoma is a population of small mature lymphoid cells with round, dense nuclei without nucleoli and very narrow rims of well-stained cytoplasm (fig. 7). Mitoses are not seen. This lymphosarcoma resembles most nearly the *human well-differentiated lymphocytic lymphoma* (*44*).

A "starry sky" pattern, associated with the presence of scattered clear histiocytes, is frequent in lymphosarcomas of cats, particularly the stem cell and lymphoblastic types. Such tumors are indistinguishable histologically from Burkitt's lymphoma of children, as pointed out by Squire (*55*) and Baskerville *et al.* (*3*). The significance of this histologic pattern is not known. But as this pattern also occurs in lymphoid tumors of other species (cattle and dogs) and in hyperplastic lymphadenopathies of non-neoplastic origin in cats, it may indicate only a rapid rate of growth and death of lymphoid cells, and/or a peculiar active phagocytosis by histiocytes, rather than implicate a specific causal agent.

Hematologic Manifestations

Leukemia, defined as a peripheral blood leukocytosis of more than 25,000 and/or circulation of immature lymphoid cells in blood, occurs in less than 25% of cats with lymphosarcoma and often appears only in preterminal stages. Holzworth (*20*) reported leukemia in 15% of cats with lymphosarcoma; Schalm (*49*), in 23%; and Crighton (*7*), in 13%. Reported variations in rates of leukemia in lymphosarcomas may merely reflect the frequency with which hematologic examinations are performed during the progression of disease and whether the animal "lives out" the full course of disease or is euthanized during relatively early stages. Gilmore *et al.* (*15*) reported that, in 10 cats with lymphosarcomas accompanied by leukemia, the leukocyte counts ranged from 5,400–220,800/mm^3, of which 16–100% were lymphocytic cells. However, leukopenia and neutrophilic leukocytosis are more frequent than frank leukemia.

The most frequently observed hematologic manifestation of feline lymphosarcoma is severe anemia that occurs in approximately one-half of affected cats. The pathogenic mechanism of the anemia, which is usually normochromic and normocytic, is not understood. One or more of the following factors may be involved: 1) replacement of bone marrow elements by lymphoid tumor cells, 2) increased erythrophagia by lymphomatous spleen and lymph nodes, 3) general inanition in advanced disease with hepatic and renal dysfunction, and 4) intercurrent feline infectious anemia (hemobartonellosis).

Anatomic Distribution

The internal viscera and lymph nodes are predominant primary sites of feline lymphosarcoma. In most cats, large lymphoid tumors are observed in organs or nodes of the abdominal cavity or of the precardiac mediastinum. When the disease is primary in the mediastinum, the tumor may enlarge to eventually fill the anterior chest cavity and encase the heart, large vessels, and esophagus, and may extend ventrally to the trachea through the anterior thoracic inlet. A frequent and valuable finding in such cases is a pleural effusion containing malignant lymphoid cells.

The kidney is usually involved bilaterally. The earliest lesions are small foci in the cortices which eventually coalesce or enlarge to form grossly visible, white knobby elevations. Extensive infiltrates can enlarge the kidneys, so that the kidneys may be palpated through the abdominal wall or seen by X rays.

The terminal ileum is a frequent primary site of feline lymphosarcoma. The neoplasm, probably beginning in Peyer's patches, forms a fusiform or cylindrical enlargement of the involved segment. The infiltrate characteristically involves all layers of the wall in a diffuse distribution so that it gradually encroaches on the lumen. The presenting symptoms are usually those of partial intestinal obstruction. The enlarged segment of the involved bowel may be palpated or seen by X ray. Four grossly similar lesions must be differentiated from intestinal lymphosarcomas: argentaffinomas, adenocarcinomas, leiomyomas, and toxoplasmic granulomas. That the ileum is an early primary site for lymphosarcoma is suggested not only by the frequent occurrence of lymphosarcoma in the ileum but also by the fact that a cure has been described after resection of an ileal lymphosarcoma (*41*).

As in other species, lymphosarcomas may involve liver, spleen, heart, lung, skin, and thymus. Two rarely observed sites of involvement are noteworthy because of their striking clinical signs: Lymphosarcoma of the larynx causes a change in quality of sound of the feline purr (*20*) and that of the spine can cause progressive paralysis (*50*).

Age

Lymphosarcoma was observed in a 6-month-old cat and several have occurred in animals between 6 and 24 months of age. Most affected cats are less than 6 years old. Occurrence at a young age is characteristic of lymphoid neoplasms of man, cattle, mice, and chickens.

In one series, lymphosarcomas involving the thymus occurred mostly in cats younger than 3 years (*20*). Crighton (*8*) reported that 3 of 4 cats with thymic lymphosarcomas were between 1 and 2 years old, which suggests an origin in thymic lymphocytes. Loveday (*31*) reported a true thymoma with epithelial elements (*54*).

The feline neoplasms show no correlation of cell type and age.

Breed and Sex

Little is known of incidence of various forms of hematopoietic neoplasms in specific breeds of cats. Dorn *et al.* (*11*) reported a slight increase in rates in Siamese cats compared to all other breeds and crossbreeds, but the difference was not significant.

The figures of Dorn *et al.* (*11*) appear to support the belief of some other investigators that male cats develop lymphosarcomas more frequently than females. In Dorn's series, lymphosarcomas accounted for one-third of all malignant neoplasms of males; in females, the rates of mammary tumors and lymphosarcomas were approximately equal (17 and 18%) and rates of both were exceeded by malignant skin tumors. It is difficult to assess the universality of the preference of male cats to lymphosarcoma, as little is known about the female-to-male ratio in the cat population in various parts of the world. Caution must be used in evaluating a possible sex predisposition of the cat to lymphosarcoma, as males usually outnumber females by about 2 : 1 in diseased cats (*20*).

Incidence

The incidence of feline lymphosarcoma cannot be determined without accurate definitions of the feline populations. Unlike the dog, the pet cat is not taxable; there is no license fee and, thus, no means for census keeping.

Dorn *et al.* attempted to define the feline population in two California counties by means of a household survey (*10*). They estimated the annual incidence of lymphomas to be 41.6/100,000 for cats—twice that for dogs in the same geographic area. That feline lymphomas occur at a rate approximately twice that of dogs is supported by a report of Jones and Berg (*26*) who found the rate of hospital admissions of cats for lymphomas to be 0.55%—twice that for dogs.

Table 2 shows relative incidence figures for lymphoid tumors and compares these figures to those for all other neoplasms in animals admitted for necropsy in three areas of the world. We feel that the Northeastern United States encompassing New York, Philadelphia, and Connecticut is one area. The relative rates range from 14.5–32% of total neoplasms. In the absence of information defining the characteristics of the animal comprising the necropsy populations from which these statistics were derived, conclusions from these comparisons would be premature and perhaps misleading.

Table 2.—Lymphoid neoplasms compared to other feline neoplasms in selected populations

Location	Investigator	Total tumors	Skin	Alimentary	Lymphoid	
					Number	Percent
London, England	Cotchin, 1952 (*5*)	226	68	66*	33	14.5
London, England	Cotchin, 1956 (*6*)	200	32	56†	39	19.0
Philadelphia, Penna., U.S.A.	Brodey, 1968 (*4*)	413	83	91	109	26.0
New York, N.Y., U.S.A.	Whitehead, 1967 (*60*)	165	26	11	46	27.0
Zurich, Switzerland	Stünzi, 1967 (*57*)	248	—	73	69	28.0
Connecticut, U.S.A.	Nielsen, 1964 (*37*)	254	75	22	82	32.0

*10 were lymphosarcomas.
†6 were lymphosarcomas.

Table 3 lists rates of feline lymphosarcomas expressed as percents of all feline necropsies from 11 laboratories in various areas of the world. It is tempting to explain the wide disparities in rates (from 2.4–9.8%) in terms of geographic differences. In fact, some investigators have noted an apparent higher relative number of lymphosarcomas in necropsied cats from urban areas compared to those derived from predominantly rural populations (*23, 42*). Evaluations of these data must await accurate definitions of the populations from which they are derived and assurance that clinical and necropsy examinations are performed with equal thoroughness and that interpretations are consistent between laboratories.

Two instances of familial or household clustering of feline lymphosarcomas have been reported. Loeb (*30*) reported lymphogenous leukemia in each of 5 kittens of a single litter, all of which died between the ages of 1 and 2 years. Schneider and associates (*51*) reported lymphoid neoplasms in 6 cats of a household of 34 cats in 3½ years. Occurrence of "clustering" in related animals or in those sharing living quarters is compatible with an infectious etiology. That individual instances of "clusters" should occasionally occur is not surprising, and these clusters reported in cats are not unlike those occasionally reported in man and in cattle.

TABLE 3.—Lymphosarcomas as percent of total feline necropsies

Investigator	Geographic location	Period	Number of necropsies	Lymphosarcomas (%)
Holzworth, 1960 (*20*)	Boston, Mass., U.S.A.	1949–60	1, 425	9. 8
Nielsen, 1964 (*37*)	Connecticut, U.S.A.	1951–63	668	9. 0
Dahme, 1968 (*9*)	Munich, Germany	1960–66	1, 281	8. 0
Head, 1968 (*19*)	Edinburgh, Scotland	1950–65	1, 626	5. 8
Dungworth, 1968 (*12*)	California, U.S.A.	1964–67	460	4. 8
Hulland, 1968 (*23*)	Ontario, Canada	1958–68	861	4. 2
Trautwein, 1968 (*58*)	Hannover, Germany	1961–67	294	3. 1
Griesemer, 1968 (*16*)	Ohio, U.S.A.	1959–67	783	2. 8
Piper, 1968 (*42*)	Washington State, U.S.A.	1963–68	551	2. 4
Stünzi, 1967 (*57*)	Zurich, Switzerland	1955–65	2, 921	2. 4
Parodi, 1968 (*40*)	Paris, France	1961–67	2, 155	2. 4

Etiology

Investigators from three laboratories have independently reported experimental transmission of feline lymphosarcomas. Intracellular C-type particles, resembling viruses of murine and avian leukemias, have been observed.

In 1964, Jarrett *et al.* (*25*) of the University of Glasgow first reported cell-free transmission of feline lymphosarcoma; they produced the disease after a latent period of 9 months. Subsequently, Rickard *et al.* of Cornell University in 1967 and 1968 (*46–48*) reported experimental transmission. In 1967, Kawakami and associates of the University of California (*27*) also reported cell-free transmission of the disease and a decrease of the latent period to 5 weeks. Viral particles similar to those of the murine Rauscher virus have been observed in plasma of cats with both spontaneous and experimental lymphosarcomas.

From the epidemiologic point of view, feline lymphosarcoma offers advantages over avian and murine laboratory models of leukemic neoplasms. The house cat is exposed to the same radioactive and chemical pollutants in homes and streets as man is, it eats similar diets, including canned foods that are sterilized and mixed with food additives, and receives drugs and vaccines prepared in the same biologic systems as those for human use.

LYMPHOID LEUKEMIA

Lymphoid leukemia is a diffuse systemic neoplastic growth of lymphoid cells. It is much less frequent in the cat than solid visceral lymphosarcomas. Holzworth (*20*) provided a comprehensive review of the literature and detailed description of typical cases. The clinical course of feline lymphoid leukemia is characterized by generalized lymphadenopathy and circulation of leukemic lymphocytes in peripheral blood. Gross lymphoid tumors of internal organs which are typical of lymphosarcomas are rarely observed in lymphoid leukemias, although frank leukemias may occur during the course of visceral lymphosarcoma.

Two forms of feline lymphoid leukemia are recognized: the lymphocytic form and the lymphoblastic form; intermediate forms between the two types have been observed.

In the lymphocytic form, the total leukocyte count may be as high as 200,000/mm^3, and the clinical course may be as long as 33 months as recorded by Loeb (*30*). Smears of peripheral blood contain a high percentage of small lymphocytes and many "smudge" cells. Bone marrow, lymph nodes, spleen, and hepatic sinusoids contain intravascular and extravascular infiltrates of relatively mature lymphocytes.

In the lymphoblastic form, the proliferating cells are large "blastic" ones with prominent nucleoli. Compared to the lymphocytic form, the lymphoblastic form usually has a lower total leukocyte count and a shorter clinical course.

Lymphoid leukemia may be difficult to distinguish from reticulosis unless hematologic studies are performed.

The lymphoblastic and lymphocytic forms of feline lymphoid leukemia are probably comparable to the acute and chronic forms of human lymphoid leukemia.

RETICULOSIS

Reticulosis, or reticuloendotheliosis, is a peculiar leukemia-like disease of cats, characterized by diffuse proliferation or infiltration of undifferentiated cells in bone marrow, peripheral blood, liver, spleen, and lymph nodes (figs. 8 and 9). In contrast to lymphosarcoma, solid tumors of viscera are not observed. Unlike the lymphocytic, monocytic, and granulocytic forms of leukemia, the cells of reticulosis, which have been called stem cells or reticulum cells, never have features that suggest differentiation toward mature hematopoietic cells of any type.

Gilmore *et al.* (*15*) first described clinical, hematologic, and pathologic features of 10 cases of feline reticulosis. A severe and persistent anemia which is refractory to therapy is invariably present and is often the first clinical sign of disease. Total blood leukocyte counts range from 6,100–53,000/mm^3 with as many as 55% of characteristic abnormal cells. In smears, the distinctive cells measure 14–20 μ, with round, often eccentrically placed nuclei, uniform nuclear chromatin distribution, and blue cytoplasm with occasional slight azurophilic granulation (fig. 10). In tissue sections, free cells have well-demarcated cytoplasmic borders and, in early or mild cases, are restricted to intravascular spaces and are easily identified in the sinusoids of the liver.

Organ distribution of infiltrates is similar to that of feline mastocytosis and of lymphocytic leukemia. Cells of reticulosis can be distinguished from mast cells by techniques that reveal metachromatic granulation. Careful hematologic examinations of bone marrow and peripheral blood will usually emphasize the distinctive characteristics of cells of reticulosis and allow differentiation from other forms of leukemia and undifferentiated lymphosarcomas.

In advanced stages of reticulosis, infiltrating or proliferating cells may be closely packed in sinusoids of the liver and may displace up to 50% of parenchyma. Varying degrees of extramedullary erythropoiesis are often associated with, or located in, the infiltrates, so that in the early phases reticulosis may be difficult to distinguish from extramedullary erythropoiesis in response to anemia.

The pathogenesis of reticulosis is unknown. The consistent association of reticulosis and anemia may indicate either that a cause-and-effect relationship exists or that reticulosis and anemia are two manifestations of hematopoietic malfunction produced by the same agent, as is seen in other species where leukemia is preceded by anemia. If the former is true, it is also possible that the high incidence of various forms of anemia in the cat and the cat's remarkable capacity for extramedullary hematopoiesis may play a role in causation of reticulosis by triggering a latent leukemogenic virus. It may be argued that the reverse is true, namely, that the reticulosis came first and that the extramedullary erythropoietic foci are a response to replacement of the destroyed bone marrow. This explanation is not completely satisfactory because no other hematopoietic tumor invading the bone marrow of the cat has been associated with the degree of extramedullary erythropoiesis that has been observed in reticulosis.

MASTOCYTOSIS

Mastocytosis in the cat is a diffuse or localized proliferation of tissue mast cells. In the diffuse form, the heaviest infiltrates are observed in the spleen, lymph nodes, bone marrow, and liver. Neoplastic mast cells circulate in peripheral blood of approximately one-half of the cats having the systemic or diffuse form of the disease. This leukemic form of mastocytosis has been confused with basophilic granulocytic leukemias of cats, and it has been variously designated in the literature as "mast cell" and/or "basophilic" leukemia. This discrepancy in terminology in the literature, particularly regarding the use of the terms "mast cells" and "basophils," and the lack of an available review of recorded cases of feline mast cell disease led to a review of pertinent literature.

In 1907, Antoine (*1*) first described splenomegaly associated with mast cell infiltration in a 13-year-old male cat. He found no gross lesions other than the spleen which was twice its normal size.

In 1931, Lillie (*29*) described mast cell leukemia in a 2.5 kg cat with splenomegaly (28 × 10 × 2.0 cm) and an enlarged mottled liver. The splenic pulp and veins and hepatic veins and sinusoids were diffusely invaded by "mast cell myelocytes." Unfortunately, blood, lymph nodes, and bone marrow were not available for examination.

In 1953, Monlux (*36*) observed an ulcerated cutaneous mastocytoma on the upper lip of a cat.

In 1956, Stünzi (*56*) described mast cell leukemia in a 14-year-old castrated male cat that died suddenly from rupture of a greatly enlarged spleen weighing 275.0 g. In addition to the spleen, the liver and most lymph nodes, particularly the mesenteric and mediastinal, were infiltrated with mast cells.

In 1957, Meier and Gourley (*34*) described mast cell leukemia in a 14-year-old castrated male cat with a total white cell count of 31,300/mm^3, of which 4% were mononucleated basophils. The spleen was 4 times its normal size and measured 25 × 8 × 4 cm; the liver and lymph nodes were only slightly enlarged. Histologically, infiltrates of mast cells were observed in the spleen, liver, lymph nodes, bone marrow, and lungs.

Also in 1957, Meier (*33*) described cutaneous mastocytomas in two 10-year-old cats. Only surgical specimens were available for study; no information on internal organs was given.

In 1958, Head (*18*) described cutaneous mast cell tumors, most of which were located on the head and neck, in 9 cats. Four of these cats developed mast cell leukemia, 3 cats at 9 years of age and 1 cat at 11 years of age. All 4 cats had heavy mast cell infiltration of the splenic red pulp and of the periportal areas of the liver.

Later, in 1962, Weiss (*59*) also described cutaneous mast cell tumors in 2 cats, 4 and 11 years old, and internal mastocytomas in 4 cats, 9½–14 years old, which involved the spleen, liver, lymph nodes, and bone marrow. The visceral mast cell infiltrates occurred primarily in organs of the reticuloendothelial system. Fewer plasma cells and eosinophils were observed in infiltrates of systemic types than in the cutaneous ones.

In 1960, Holzworth (*21*), in an excellent review of leukemic malignancies of cats, mentioned a basophilic leukemia in a cat with an extremely enlarged liver. The leukocyte count ranged from 24,000–31,000/mm^3 with 4–7% basophils.

Also in 1960, Labie and Fontaine (*28*) described 3 cats, aged 4, 5, and 7 years, with enlarged abdomens, frequent vomiting, and other evidence of gastrointestinal disturbances. All 3 cats had splenomegaly associated with mast cell infiltration. Diagnoses were established in 2 cats by splenectomy and, in the third cat, by percutaneous needle biopsy. None of these 3 cats had cutaneous involvement.

In 1961, Quaglio and Testi (*43*) described an enlarged liver, lymph nodes, and spleen (25 × 8 cm) associated with diffuse infiltrations of mast cells in a cat.

Later, in 1965, Schalm (*49*) described an 8-year-old cat with a distended abdomen, history of weight loss, and a leukocyte count of

30,000/mm^3, 35% of which were mast cells. Necropsy revealed splenomegaly, white foci in the liver, and a large gastric ulcer. Microscopically, mast cells were in spleen, lung, and lymph nodes, and they accounted for 6.3% of the nucleated cells in the bone marrow.

In 1964, Seawright and Grono (*52*) described a 9-year-old male cat with "several hundred" cutaneous mast cell tumors, hepatomegaly (liver twice its normal size), splenomegaly (spleen 10 times its normal size), and a perforated duodenal ulcer which was the ultimate cause of death. Histologic examination of spleen, liver, and subcutaneous lymph nodes revealed invasion by neoplastic mast cells, as did cross sections of blood vessels throughout the body.

Also, in 1964, Lucke (*32*) described hepatomegaly and splenomegaly in a 12-year-old spayed female cat. The 380 g liver and the 76 g spleen contained white foci on cut surfaces. Histologic examination revealed mast cell infiltrations of the liver, spleen, bone marrow, lungs, and in renal blood vessels.

In 1964, Nielsen (*37*) described a cutaneous mastocytoma in a 10-year-old cat with metastasis to the regional axillary lymph node.

In 1965, Hauser (*17*) described mast cell leukemia in an 11-year-old spayed female cat, with a history of pruritic eczema over the entire body, and a total leukocyte count of 52,500/mm^3, 60% of which were mast cells. Lymph nodes and spleen were markedly enlarged. Skin, liver, myocardium, pancreas, lung, and bone marrow were diffusely invaded with mast cells.

Also, in 1965, Ohshima and Miura (*39*) described mast cell leukosis in 2 cats. One cat, a 16-year-old female, died with clinical signs of inappetence and a distended abdomen. The liver was enlarged (210 g). The spleen was also enlarged (45 g) and measured 21.5 × 4.5 × 2.0 cm. Liver, spleen, and mesenteric nodes were diffusely infiltrated with mast cells, in which red-violet granules were observed with cresyl violet stains. One-third of the cells contained oxidase-positive granules. The other cat, a 12-year-old female, died with clinical signs of anemia and splenomegaly. At necropsy, the liver was enlarged (170 g). The spleen weighed 175 g and measured 22 × 6 × 2 cm and had rounded margins and a brick-red cut surface. Histologically, the mast cell infiltrates were similar to those observed in the first cat.

In 1968, Ishmael and Howell (*24*) described splenomegaly in an 8-year-old castrated male cat with a clinical history of anorexia and vomiting for 3 months. Splenectomy revealed a greatly enlarged spleen measuring 22 × 7 × 2.5 cm associated with mast cell infiltrates.

Information from this review and examination of material from 11 cases in the files of the Department of Animal Diseases of the University of Connecticut are the basis for the following characterization of feline mast cell disease:

Feline mastocytosis is predominantly a systemic neoplastic growth of mast cells which characteristically involve hematopoietic organs including spleen, liver, lymph nodes, bone marrow, and peripheral blood (figs. 11 and 12). In contrast to the skin of the dog, feline skin is rarely affected.

Splenomegaly, often massive, is characteristic of feline mastocytosis, and the term "splenic mastocytosis" has been used to describe the feline disease. Massive splenomegaly is considered by some practitioners to be almost pathognomonic of mastocytosis, although other causes of splenomegaly exist.

Several cats with mastocytosis have had evidence of disturbed gastrointestinal motility, including vomiting, associated with excessive production of histamine by mast cells. A few cats have had ulcers of gastric and duodenal mucosa, similar to those described in dogs with histaminosis associated with advanced mast cell neoplasia.

The organ distribution of feline mastocytosis is different from that of generalized mastocytosis of dogs. In the dog, cutaneous involvement is invariably present and the skin appears to be the primary site of mast cell proliferation. In the cat, the skin is rarely affected; the disease appears to originate in the hematopoietic system. Because the distribution of mastocytosis is similar to that of reticulosis, some German investigators have considered mastocytosis to be a form of reticulosis with mast cell differentiation and used the term "mast cell reticulosis." The resemblance of mastocytosis and reticulosis is also supported by the relatively infrequent occurrence of internal tumor masses and the leukemic blood changes observed in both diseases. In mastocytosis, the mast cell infiltrates in the liver and in bone marrow tend to occur in discrete spherical foci remarkably uniform in size and shape—unlike the diffuse cellular infiltrates of reticulosis and lymphoid leukemia which are intravascular, and invariably, even in advanced stages, form irregularly shaped infiltrates, usually with portal triads as the centers.

To distingush mastocytosis from reticulosis is not difficult if smears, imprints, or properly fixed sections are available to demonstrate the metachromasia of mast cells (fig. 13). Postmortem lysis of mast cell granules occurs readily and these granules are soluble in water. Mast cells with lysed granules and reticulum cells resemble each other; both have nuclei similar in size and in staining characteristics and similar nucleus-to-cytoplasm ratios.

One problem in recognition of mastocytosis has been the tendency, indicated in reports in the literature, to confuse feline mastocytosis and basophilic leukemia. It is likely that true basophilic leukemia does occur in the cat but apparently it is extremely rare. From illustrations and descriptions in reports in the literature, it appears that most, if not all, reported feline basophilic leukemias were actually diffuse or leukemic forms of mastocytosis. Mast cells in peripheral blood or bone marrow, stained with toluidine blue, May-Grünwald-Giemsa, or Wright's, may be distinguished from blood basophils by the following criteria: 1) Mast cells are larger—they average 25 μ, whereas basophils average only

15 μ; 2) unlike the segmented or lobulated nuclei of mature blood basophils, nuclei of mast cells are round or ovoid; and 3) metachromatic granules of mast cells are larger and more numerous than those of blood basophils, which often contain only a few rod-shaped, well-spaced granules. These differential criteria may not be so obvious if immature or myelocytic forms of blood basophils are involved in the leukemic process.

MYELOPROLIFERATIVE NEOPLASMS

Myeloproliferative neoplasms are rare in cats compared to those of lymphoid origin. Table 1 lists the forms that have been observed in cats.

Granulocytic Leukemias

Granulocytic leukemias are the most prevalent myeloproliferative leukemias of cats; yet less than 20 convincing examples have been reported (*2, 13, 14, 21, 30, 35, 45, 49*). Two examples were designated specifically as eosinophilic leukemias (*21, 53*); basophilic leukemias have been confused with disseminated mastocytosis and are discussed in another section of the paper.

Immature leukemic granulocytes are often difficult to distinguish from immature lymphocytes, monocytes, or reticulum cells. Peroxidase stains are sometimes helpful in these cases. Alkaline phosphatase, present in mature granulocytes of most species, including man, cannot be demonstrated in neutrophils of the cat (*61*), and this reaction cannot be used to distinguish leukemoid reactions from granulocytic leukemia in the cat.

The features of the feline disease are similar to those of granulocytic leukemia of man and other species. Liver, spleen, and sometimes lymph nodes are enlarged. Leukocyte counts may be as high as 300,000/mm^3. Immature granulocytes are numerous in bone marrow and diffusely infiltrate liver, spleen, and other organs. Chromosomal abnormalities have not been reported in cats.

The largest reported series was that of Holzworth (*21*) who described granulocytic leukemia in 9 cats. Ages ranged from 1–10 years. Clinical signs, present from a few days to 3 weeks, included depression, weakness, inappetence, and fever. Most affected cats had a palpable liver and spleen, and a few had enlarged superficial lymph nodes. All cats had severe anemia, with hemoglobins in the range of 2.0–5.0 g/100 ml. In 3 cats, leukocyte counts were subnormal. In the other cats, they were elevated, the highest being 65,000/mm^3. Myelocytes with round, often eccentrically located nuclei and deep-blue cytoplasm with reddish granules were observed in peripheral blood. The granules were peroxidase positive. At necropsy, spleens and livers were 2 or 3 times their normal size. Some cats had one or more lymph nodes involved. Bone marrows were hypercellular, and myelocytes or myeloblasts predominated. Increased numbers of megakaryocytes were observed in the marrow of some cats. Leukemic cells diffusely infiltrated kidneys, heart, and lungs, were particularly abundant in portal areas and sinusoids of liver, and were diffuse in red pulp of the spleen. Megakaryocytes and erythropoietic foci were observed in livers and spleens of some cats.

The granulocytic leukemia described in a 4½-year-old cat by Reid and Marcus (*45*) was characterized by a blood leukocyte count of 369,000/mm^3, with 69% myeloblasts, a hematocrit of 30%, and diffuse infiltrations of liver, spleen, and lymph nodes by leukemic cells.

Eosinophilic Leukemia

Eosinophilic leukemia is rare in cats and only 2 convincing cases have been reported (*21, 53*).

One was an 8-year-old castrated male, with weight loss, emesis, diarrhea, hepatosplenomegaly, anemia, and a leukocyte count of 150,000/mm^3, with 46% segmented eosinophils and 22% band eosinophils (*21*).

The other was a 4-year-old, spayed, tricolor female, with bloody stools and leukocyte counts ranging from 88,700–136,000/mm^3, with 82% segmented eosinophils and 35% band eosinophils, respectively. Tumorous eosinophilic infiltrates were seen in bone marrow, kidneys, lymph nodes, liver, spleen, heart, and intestines. In the colon, the infiltrates were so severe that ulcers developed (*53*).

Erythroleukemia

Schalm (*49*) described erythroleukemia in 2 cats, aged 5½ and 7 years. Nucleated erythrocytes accounted for 65 and 49%, respectively, of the leukocytes. The first cat had severe anemia, without reticulocytes in the blood but with many giant metarubricytes and unclassified cells with deeply basophilic cytoplasm and eccentric nucleus. Necropsy revealed infiltration of spleen, liver, and lymph nodes with cells varying in size from large, irregular myeloblasts to small, dark-staining cells.

Zadwidzka *et al.* (*62*) described in detail the hematologic and necropsy findings in a 5-year-old castrated male cat with erythremic myelosis, resembling Di Guglielmo's syndrome in man. The erythrocyte count was 1 million/mm^3, the hemoglobin 2 g%, the packed cell volume 5%, the leukocyte count ranged from 4400–8500/mm^3 with 10.5% band cells. The number of nucleated red cells per 200 leukocytes in the peripheral blood was: rubriblasts 5, prorubricytes 18, rubricytes 39, and metarubricytes 28. The bone marrow differential count showed 26.8% rubriblasts and 14.6% prorubricytes.

Monocytic Leukemia

Holzworth (*21*) described 2 cats, aged 3 and 6 years, having leukocyte counts within normal range with 45 and 29% monocytes. Supravital studies were used to establish a diagnosis of monocytic leukemia in the first cat. At necropsy, this cat had marked hyperplasia with numerous mitoses of reticulum cells in lymph nodes, spleen, and liver. The reticulum cells had round hyperchromatic nuclei with variable numbers of nucleoli and showed differentiation toward monocytes and macrophages with phagocytic ability. Clinical features of the second cat were similar, but necropsy was not described.

Schalm (*49*) described a 4-year-old male cat which had anorexia, depression, weight loss, emesis, and pyrexia for a 2-week period. The leukocyte count during the course of the disease decreased from 13,800 to 4,000/mm^3, with monocytes ranging from 0–25%. "Atypical" cells accounted for 1–39.5%. Necropsy revealed the mesenteric nodes to be enlarged 3 or 4 times; leukemic cells were present in liver, heart, and kidneys.

Plasma Cell Neoplasms

The only example of this neoplasm in the literature was that reported by Holzworth and Meier (*22*). A 7-year-old castrated male Persian cat had exhibited lethargy, inappetence, wasting, and a slowly progressing anemia. Hemograms revealed a total leukocyte count ranging from 1,650–13,400/mm^3, with 10% of large mononuclear cells, many with eccentric nuclei, marked rouleaux formation of erythrocytes, and a hemoglobin of 4.1–7.0 g. Serum electrophoresis revealed an increase in γ and α-2 globulins. Urinalysis revealed a specific gravity of 1.017 and a 2+ albumin. Bence Jones protein was not present. At necropsy, the spleen was 3 times its normal size and the cervical nodes were twice their normal size. Histologic studies revealed foci of immature plasma cells in the marrow and diffuse infiltration in the lymph nodes, spleen, and liver.

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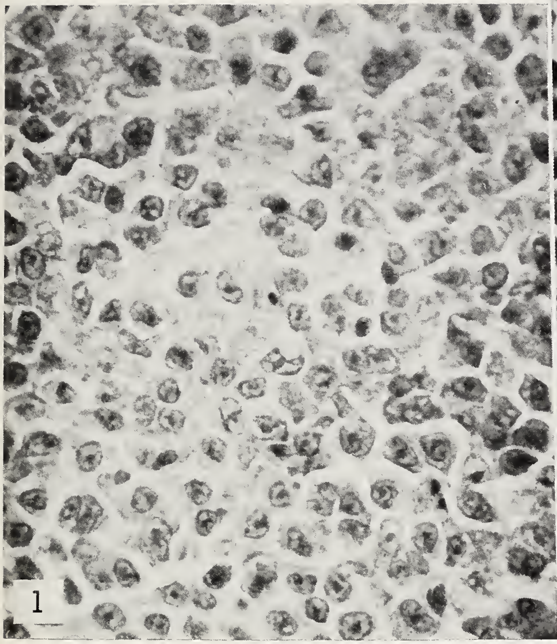
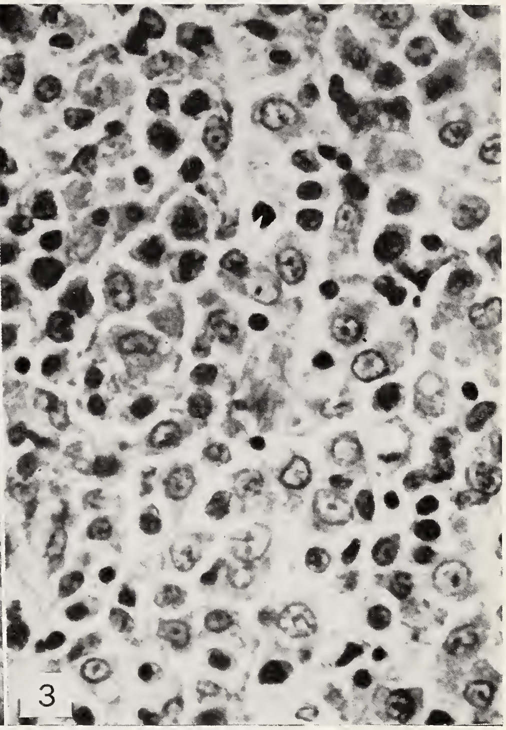




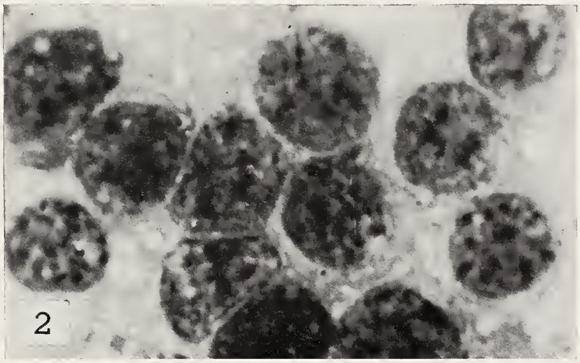
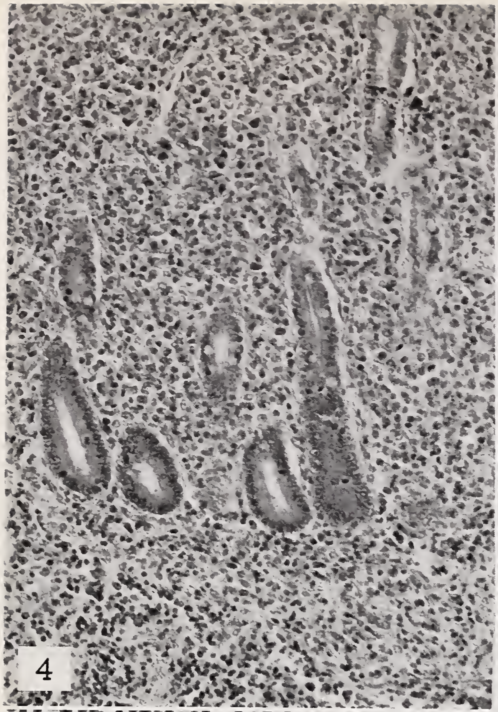
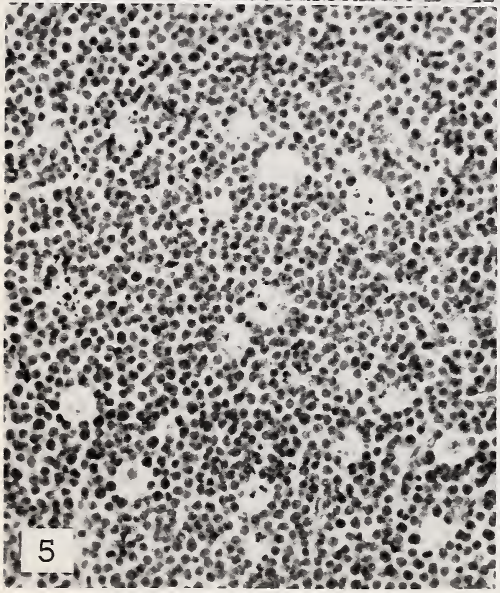


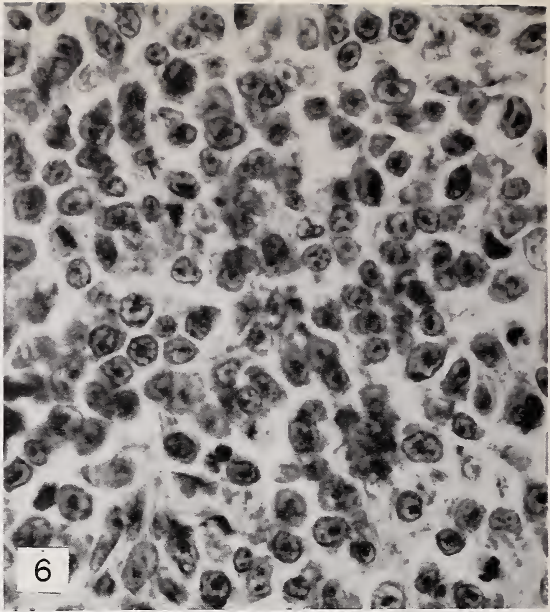
Figure 1.—Stem cell lymphosarcoma. This undifferentiated lymphosarcoma is a uniform population of relatively large cells with vesicular nuclei and rather prominent nucleoli. Hematoxylin and eosin. × 500

Figure 2.—Impression smear of mesenteric nodule infiltrated with stem cell lymphosarcoma. Giemsa. × 1200

Figure 3.—Pleomorphic lymphosarcoma of kidney showing mixture of neoplastic histiocytes and lymphoid cells. Hematoxylin and eosin. × 500







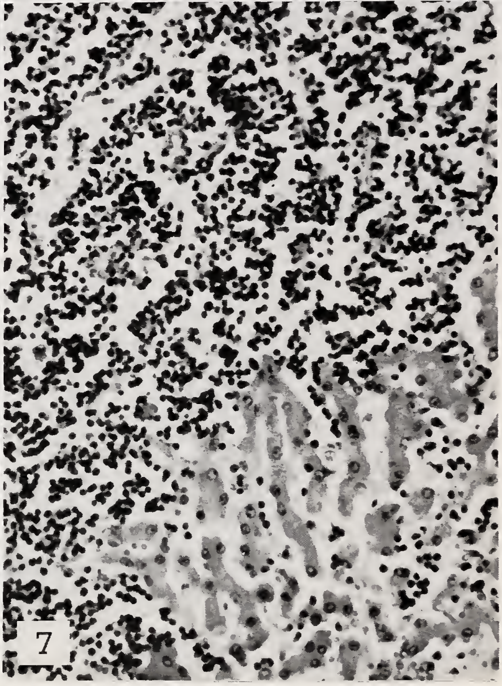
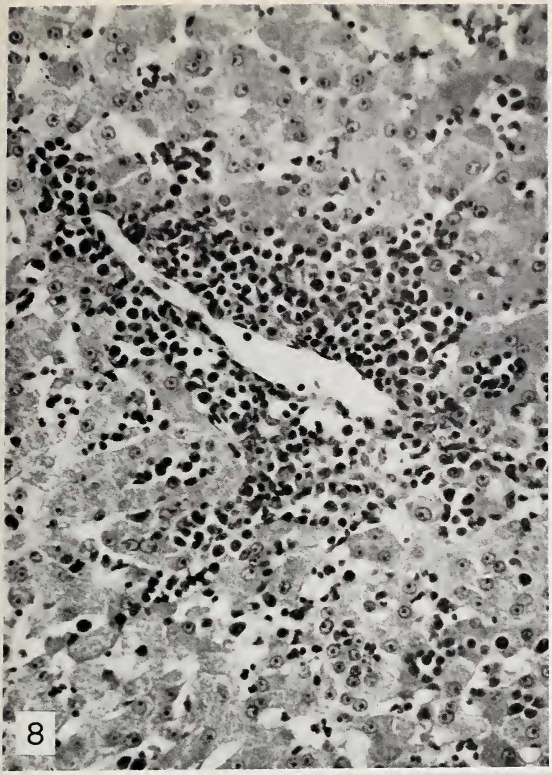


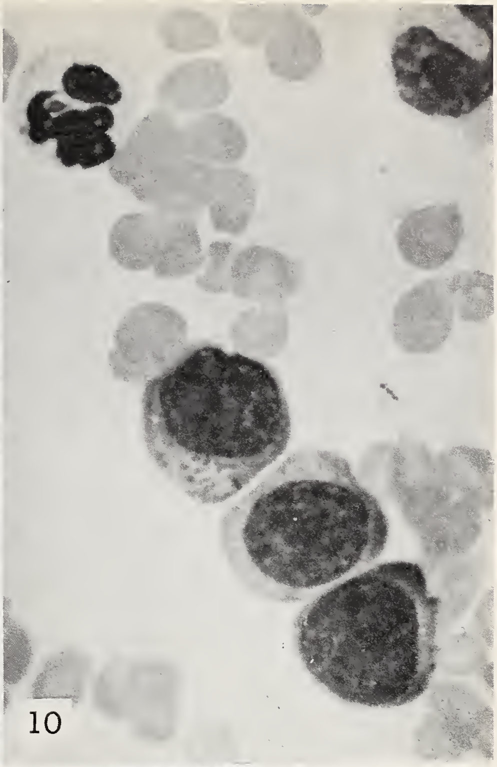
Figure 4.—Lymphoblastic lymphosarcoma of ileum. Hematoxylin and eosin. × 200

Figure 5.—Lymphoblastic lymphosarcoma of lymph node with several clear phagocytic histiocytes giving a "starry sky" appearance. Hematoxylin and eosin. × 200

Figure 6.—Lymphoblastic lymphosarcoma of kidney illustrating uniform proliferation of lymphoblasts with vesicular nuclei having large prominent nucleoli and many mitoses. Hematoxylin and eosin. × 500

Figure 7.—Lymphocytic lymphosarcoma of liver. Hematoxylin and eosin. × 200





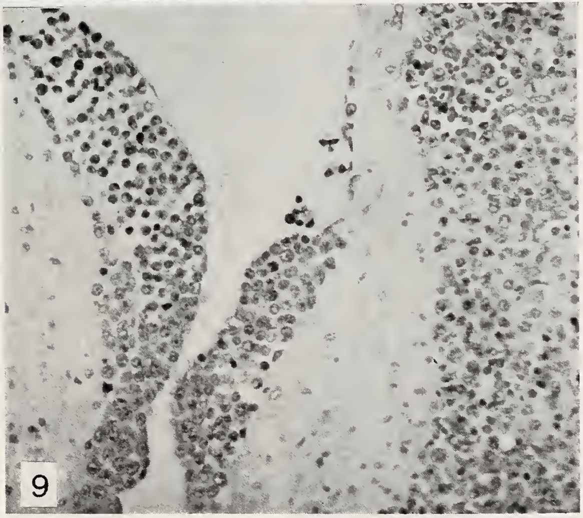
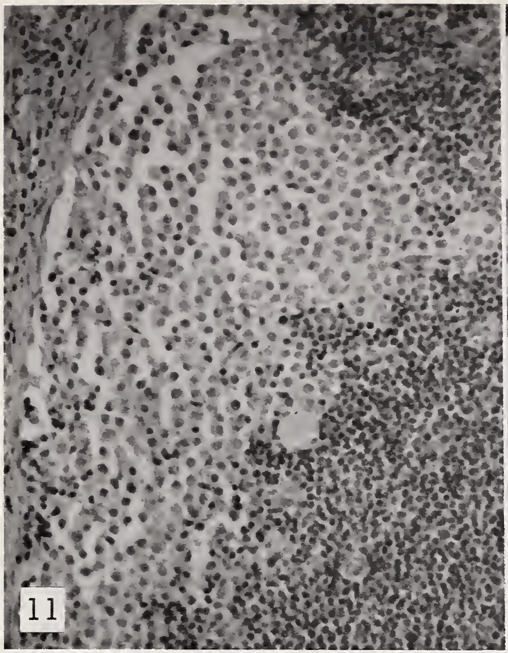
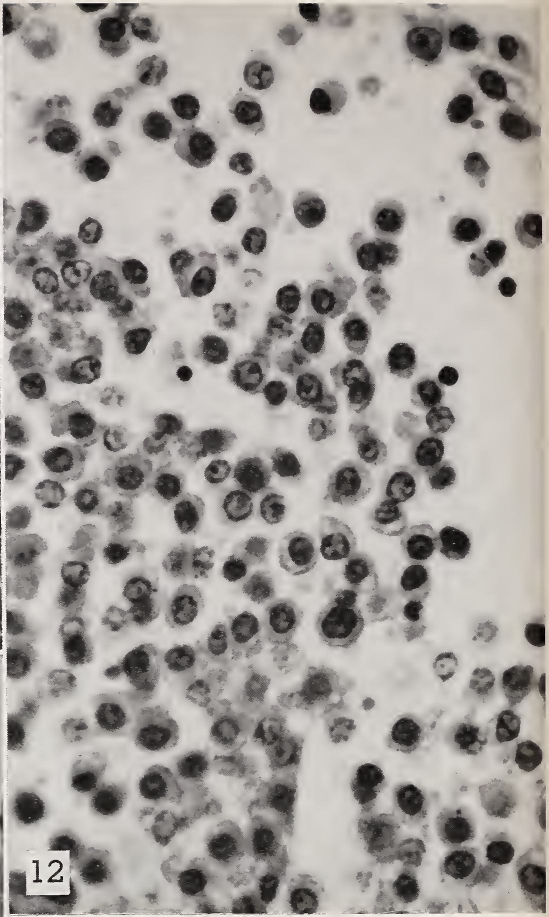


Figure 8.—Liver of cat with early reticulosis showing centrolobular infiltrate of reticulum cells, normoblasts, and lymphocytes. Hematoxylin and eosin. × 260

Figure 9.—Splenic trabecular vessel with massive subintimal infiltrate of reticulum cells in reticulosis. Hematoxylin and eosin. × 400

Figure 10.—Blood smear, reticulosis, showing characteristic undifferentiated cells. Giemsa. × 1200





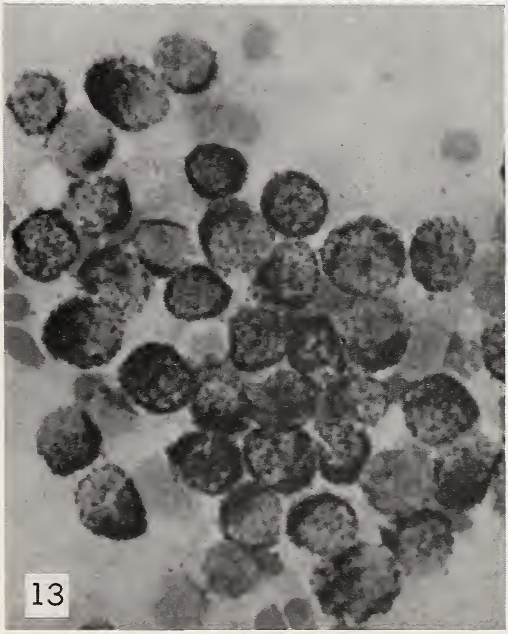


Figure 11.—Neoplastic mastocytes in marginal sinus of lymph node. Hematoxylin and eosin. × 300

Figure 12.—Mastocytosis, lymph node, illustrating free tumor mast cells with round, centrally placed nuclei in abundant cytoplasm and well-demarcated cellular borders. Hematoxylin and eosin. × 600

Figure 13.—Impression smear of mastocytes from spleen of cat with diffuse mastocytosis showing heavily granulated neoplastic mast cells and a few red cells. Giemsa. × 1200

Chromosomal Analyses of Feline Lymphomas[1,2]

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CYTOGENETIC STUDIES of 23 cases of feline malignant lymphoma revealed a panoply of chromosomal anomalies among the malignant cells. Aneuploidy was the dominant feature and occurred in varying degrees, including loss of whole chromosomes and replication of other chromosomes. Diagnoses in all cases were confirmed by microscopic examination of tissues at necropsy or, in a few cases, by examination of tissues removed surgically. The karyotypes of each tissue examined were arranged according to the San Juan system. For convenience, the cases have been arranged in five groups according to the predominant anomaly, although occasionally some cells were found with chromosome numbers falling in two groups.

Group I.—The modal number of chromosomes in abnormal cells was 37 (the normal 2n number for *Felis catus* is 38). This reduced number was due to the loss of a single chromosome in group E. Three cases were in this category.

Group II.—The modal number of chromosomes was 37. This reduced number was due to the loss of a single chromosome in group D. Nine cases were in this category.

Group III.—The modal number of abnormal cells was 39. The additional chromosome was due to trisomy of a chromosome in group F. Five cases were in this category.

Group IV.—Chromosome numbers were 40 and 41. This aneuploid group was the result of additional chromosomes in the C, D, E, and F groups. Two cases were in this category.

Group V.—No defect in chromosomes or their number was found. Four cases were in this category.

The significance of this panoply of chromosomal aberrations is not understood nor is the relationship of this aneuploidy to the malignant behavior of the cells established. Studies are being continued to correlate these chromosomal abnormalities with other features of the diseases and will include distribution of lesions, cell morphology, biological behavior, age and sex of the animals, etc.

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[1] Presented, in part, at the Mammalian Cytology and Somatic Cell Genetics Conference, Williamsburg, Va., 1965.

[2] Dr. C. E. Gilmore, Nancy Mah, and Ming Ming Hu also participated in these studies.

Spontaneous Hematopoietic Tumors of Dogs [1, 2]

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LYMPHOMAS

IN THIS paper the term lymphoma is used in its broadest sense to indicate all primary tumors of the lymphoid or immune system, and it includes both the sarcomatous and leukemic manifestations.

Lymphomas are among the most prevalent neoplasms of dogs. One recent report indicated an estimated annual incidence of 24 per 100,000 dogs (*1*). The only other canine tumors that occur with greater frequency are those of the mammary gland and mastocytomas and histiocytomas of the skin.

Lymphomas occur most frequently in mature dogs, between 5 and 10 years old, although they may occur at any age. Purebred dogs appear to be more frequently affected. Our data indicate an apparent excess of lymphomas in boxers and cocker spaniels even though the popularity of these breeds has declined considerably in recent years. Unlike human lymphomas in which males predominate, a sex predilection for these neoplasms has not been noted in dogs. However, it must be remembered that many female dogs have undergone oophorectomy before sexual maturity and comparisons of relative rates of tumor incidences to those of humans may not be strictly valid.

Although the importance of canine lymphomas in clinical veterinary medicine is generally acknowledged, their value as biomedical models of spontaneous neoplastic disease is not generally appreciated by veterinary or medical investigators (*2*) who prefer naturally occurring or induced tumors of laboratory small rodents for most research in etiology and therapy of neoplasms. Advantages of mice include genetic definition, con-

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] This study was supported in part by Public Health Service grant FR 000130-04 from the Division of Research Facilities and Resources and training grant GM 00415 from the National Institute of General Medical Sciences, and U.S. Army contract D18-064-ANS-104 (A) Task D.

[3] I wish to acknowledge the technical assistance of Miss Carole Heckner and Mr. Robert Knight and the clerical assistance of Miss Johnnetta Perkins. The photography was by Mr. Peter Lund.

venience, and availability, but inbred mice are not in all respects the most appropriate models of human neoplastic diseases. The genetic uniformity, relatively short lifespans, and yet undefined host factors involved in artificial induction or transplantation of tumors preclude completely realistic comparisons to human diseases. Extensive supportive clinical studies of rodents are impractical because of their small size. The naturally occurring lymphomas of dogs compare well with some human counterparts in their clinical and pathologic characteristics and their responses to chemotherapy. In addition, genetic heritages and environmental exposures of dogs are more nearly comparable to those of man. The size of dogs, their tractability, and relatively long lifespan allow application of virtually all clinical procedures and forms of therapy used in human medicine. The recent evidence which suggests viral etiologies for canine lymphomas (*3–5*) certainly enhances their potential attractiveness as experimental subjects.

Characteristics of canine lymphomas have been described in several reports (*6–16*). This paper records preliminary results of personal observations of more than 70 dogs with lymphomas, in which extensive clinical, pathologic, and cytologic studies were performed. Animals with suspected lymphomas are referred to us by practicing veterinarians. Diagnoses are confirmed by biopsy. Tumors are classified according to patterns of nuclear chromatin, enzyme activity, and cytoplasmic pyroninophilia. Animals are followed by periodic clinical examinations during therapy until termination. Repeat biopsies are performed periodically. All observations are finally correlated with responses to therapy. These will be summarized in descriptions of *Pathology* and *Therapy*.

Lymphosarcoma

CLINICAL FEATURES

The most frequent form of lymphoma in dogs is lymphosarcoma. The initial sign is usually painless lymphadenopathy (fig. 1). Owners often report that the initial sign is enlargement of lymph nodes about the head and neck, but widespread involvement of peripheral nodes and, occasionally, the tonsils is often noted when the animal is first presented to a veterinarian. The nodes usually enlarge rapidly and may be matted together. Splenomegaly may also be detected. Involvement of peripheral blood, bone marrow, and other viscera usually occurs in later stages of disease. In fewer cases, internal nodes or visceral structures are primarily involved and the diagnosis is less obvious. The usual presenting picture of widespread involvement limited to lymphoid tissues is equivalent to Stage III in Hodgkin's disease of man (*17*). Nonspecific signs of systemic involvement noted in early stages of fewer than half of the animals include lethargy, anorexia, fever, anemia, and weight loss.

Any tissue or organ of the body may be affected eventually. Later manifestations which depend on sites of involvement include gastrointestinal disorders, respiratory distress, uremia, anemia, and icterus. Two dogs of our series first presented with paraplegia; neither had obvious lymphadenopathy. One dog had diffuse tumor infiltration of several vertebrae and multiple pathologic fractures but despite marrow involvement did not have leukemia. The other dog had multiple epidural masses of tumor and leukemia. Paraplegia or other paralyses incident to infiltrations of the spinal canal are relatively common in bovine lymphosarcoma but there are apparently no other reports of this phenomenon in dogs.

Leukemia, determined by the presence of primitive or atypical cells in peripheral blood, occurs late in the disease in more than 50% of dogs with lymphomas and is usually associated with extensive tumor dissemination. If leukemia develops, the total leukocyte count may be markedly increased but, more frequently, there is a relative lymphocytosis with a normal or only slightly elevated total count. The cells are usually lymphoblasts with uniform, finely particulate, and evenly dispersed nuclear chromatin without condensation or clumping, and nucleoli may or may not be visible (fig. 2). The cytoplasm is relatively inconspicuous or occurs as a distinct basophilic rim. The nucleus is often eccentrically placed with a pale perinuclear Golgi area. Frequently, such cells appear very fragile, and distorted or smudged forms are numerous. Occasionally, primitive stem cells or histiocytes with broader cytoplasm and prominently stippled chromatin predominate (fig. 3). Less frequently, atypical prolymphocytes with some degree of chromatin condensation, large irregular nuclei, and prominent nucleoli predominate. These cells are similar to circulating lymphosarcoma cells sometimes observed in the human disease (*18*, *19*).

In a very few cases, the canine disease presents as a leukemia with generalized, mild lymphadenopathy and hepatosplenomegaly. In these instances, it is difficult to determine whether the disease is acute lymphocytic leukemia or lymphosarcoma; both diseases may be clinical variants of a similar underlying process in dogs as in other animals and in man. The pathogenic features of the canine disease point to such a relationship. Although the usual clinical presentation in the dog is that of lymphosarcoma, the high incidence of wide dissemination and leukemia suggests that the canine disease combines features of both lymphosarcoma and acute lymphocytic leukemia. Counterparts of human chronic lymphocytic leukemia or lymphosarcoma consisting of well-differentiated lymphocytes are apparently rare in the dogs, and I have seen only 3 animals with this type of cell population.

A frequent hematologic feature of canine lymphosarcoma is a granulocytic leukemoid reaction which may be of a degree suggesting granulocytic leukemia. The cause of this marrow response is unknown. It does not appear to be a response to sepsis. Primitive cells, including myelocytes, promyelocytes, and even myeloblasts, may be numerous in the peripheral blood. Large cells with indented or folded nuclei, delicate lacy chromatin patterns, and faintly granular blue-gray cytoplasm are often present. These appear to be monocytes and are apparently part of the nonspecific response. The bone marrow shows myeloid hyperplasia, but extramedullarly myeloid metaplasia does not occur. This leukemoid reaction is responsible for some erroneous diagnoses of granulocytic leukemia in dogs in the earlier literature. When true lymphocytic leukemia occurs with a leukemoid reaction, this creates a very striking and confusing peripheral blood picture.

Anemia and, less frequently, thrombocytopenia are observed particularly in leukemia with marrow replacement. Some affected animals also show progressive hemolytic anemia although there have been no reports of positive direct Coombs' tests. In such cases, peripheral smears show normocytic, normochromic erythrocytes with spherocytes, target cells, and reticulocytes. Dogs with lymphosarcomas frequently have elevation of serum γ-globulins. We have seen hypogammaglobulinemia in one animal with extensive involvement of the thymus.

Length of survival after diagnoses indicates that canine lymphosarcomas are acute diseases in young or middle-aged animals but tend to be relatively chronic in older animals, a pattern consistent with that observed in the human diseases. Animals with lymphomas are rarely treated and most are subjected to euthanasia before natural death occurs. Thus there is little information concerning effects of therapy on longevity. In our studies, the animals are allowed to survive until terminal stages. We estimate that survival times in untreated middle-aged animals average less than 6 months after onset of disease. For both treated and untreated animals younger than 2 years, survival is often less than 2 months. In the few lymphomas that we have observed in animals more than 12 years of age, survival times have extended over 6 months without treatment. In dogs, response to therapy and longevity cannot be accurately correlated with clinical staging because most animals are in Stage III when first presented for diagnosis. We have found that response to therapy correlates with certain cytologic features of individual lymphomas; this will be discussed in detail under Therapy.

Pathologic Features

As in other species, lymphomas of dogs may manifest as diffuse or nodular masses in a variety of organs or tissues. Lymphomas are considered to be of unifocal origin with metastases occurring secondarily. Primary leukemias which appear to be rare in dogs are believed to develop multicentrically, and widespread infiltrates are expected to occur early during the diseases. In advanced lymphosarcomas of man and animals, morphologic pictures identical to leukemia are often seen and it may be difficult to distinguish lymphoma from leukemia. This is often observed in dogs in which wide dissemination and leukemia are frequent.

In terminal stages of lymphomas of dogs, virtually all groups of peripheral and internal lymph nodes are affected. These nodes may be extremely large and matted together. Histologically, the architecture of nodes is replaced by a monotonous population of tumor cells. Other organs commonly involved are spleen, liver, bone marrow, tonsils, kidneys, lungs, gastrointestional tract, pancreas, heart, and eyes—in approximately that order. The nervous system, reproductive system, and endocrine tissues are less frequently affected.

Splenomegaly may be severe and may be the primary cause of distress of a dog (fig. 4). Splenic involvement originates in the lymphoid nodules and spreads diffusely to obliterate pulp cords and sinuses. Less often discrete tumor nodules protrude through the capsule of the spleen although there is little or no diffuse enlargement.

In the liver, the periportal areas are affected most constantly, but sinusoidal and centrilobular infiltrates are also frequent, especially if leukemia is present (fig. 5). The portal infiltrates impart a characteristic pale lobular pattern to the gross appearance of the organ.

Infiltrates of kidneys may be diffuse or nodular, and kidneys may be sites of primary lymphosarcomas although not nearly as frequently as in cats. Because chronic interstitial nephritis characterized by lymphocytic and plasma-cellular infiltrates is common in older dogs, close examination may be necessary to distinguish infiltrates of lymphomas from inflammatory cells.

Discrete tumor masses may be present in lungs, but generalized perivascular and peribronchiolar infiltrates are more frequent.

The intestine, particularly the ileum, is occasionally involved by annular or nodular tumors and is one of the more frequent extranodal sites for primary lymphomas. Origin appears to be Peyer's patches.

Compared to incidence in other animals, anterior mediastinal or thymic involvement is rare in dogs. It has seldom been reported, and we have seen only 3 examples.

The true incidence of involvement of bone marrow is difficult to determine because marrow is not always examined in clinical and pathologic examinations. In our material, involvement of marrow has correlated well with the presence of leukemia, *i.e.*, slightly more than 50% incidence, and is observed primarily in later and more advanced stages of disease. Tumor cells may be in focal aggregates rather than diffuse infiltrates and, therefore, may be missed in marrow taps or biopsies.

Microscopic infiltrations of structures of the eye, particularly the iris-ciliary body, and the third eyelid and its glands, are not infrequent. Blindness from infiltrates of tumor or from resulting edema or glaucoma is rare.

CYTOLOGY

In routine fixed sections, cells of most canine lymphomas are relatively undifferentiated and similar to those of stem cell types of human lymphomas. Nuclear membranes are distinct but delicate. Nucleoli and chromatin particles are prominent, and cytoplasm is indistinct (fig. 6). Less frequently the cells are smaller with coarse nuclear membranes (fig. 7). They are irregular in shape, slightly larger than small lymphocytes, and correspond most nearly to those of poorly differentiated lymphosarcomas of man.

Tumors classified as histiocytic types are relatively uncommon in dogs. When observed, they are composed of bizarre reticulum cells with large and often irregular nuclei and heavy nuclear membranes. The cytoplasm is more eosinophilic than basophilic and is broader and more branching than that of lymphocytes, although lymphocytes may be numerous in infiltrates. Descriptions based on fixed sections alone, which include many artefactual features produced by fixation, do not allow critical and consistent classification of cells. For example, in our experience, many tumors classified as reticulum cell sarcomas from examination of fixed sections are in fact, lymphosarcomas with bizarre cytology when fresh tumor imprints are examined.

Two cytogenetic studies of canine lymphomas have been reported (*20, 21*). Both have indicated that abnormal karyotypes occur, but that there were no specific changes common to all cases.

We have studied various cytologic features of the canine disease and have correlated our results with clinical courses and response to chemotherapy. The procedures are performed on air-dried, fixed or unfixed fresh tumor imprints. Tumors are classified according to chromatin morphology, cytoplasmic pyroninophilia, and hydrolytic enzyme activity. Predominant cells are grouped according to chromatin morphology as poorly differentiated (histiocytes and lymphoblasts) or well-differentiated (prolymphocytes or lymphocytes). Most tumors are either lymphoblastic or prolymphocytic. The criteria for cytologic classification have been described previously (*22*). Briefly, the least differentiated cells or histiocytes have large, irregularly shaped nuclei often with prominent nucleoli. Chromatin particles are coarse and distinct but not clumped and present a prominent stippled appearance (fig. 8). Lymphoblasts have more regular, round nuclei and very fine chromatin particles without clumping, resulting in a uniform, "ground glass" appearance (fig. 9). Prolymphocytes show some evidence of chromatin condensation with variable areas of dark clumping and intervening pale, uniform chromatin (fig. 10). Nucleoli may or may not be visible in lymphoblasts or prolymphocytes. Lymphocytes are smaller and show more condensation of chromatin.

Pyroninophilia, which is completely ribonuclease digestible, is graded as strong or weak with the use of standardized, reproducible staining methods[4] (figs. 11 and 12). Enzymes include acid phosphatase (*24*), nonspecific esterase[5], and β-galactosidase (*25*). Activity is evaluated in a semiquantitative manner by counting numbers of positive cells in 10 high-power fields of imprint monolayers (fig. 13). These procedures are relatively simple, and results are reproducible. The positive cells are scattered macrophages with strong lysosomal activity, most of which cannot be recognized by routine histologic procedures. The biochemical activity may represent a crude evaluation of the degree of spontaneous tumor cell death, the same phenomenon represented by the familiar "starry sky" pattern, and may reflect an immunologic response by the host.

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[4] The procedure was a modification of the method described by Barka and Anderson (*23*).

[5] The procedure was the naphthyl acetate method recommended by Barka and Anderson (*23*).

These procedures can be rapidly performed on imprints of biopsied material and could be adapted easily for use in any diagnostic pathology laboratory.

Therapy

Lymphomas of dogs undergo partial or complete remissions in response to chemotherapy. Clinical therapeutic regimens based on our experimental procedures have been recommended for use by practicing veterinarians (*26*).

We have treated 45 dogs with lymphomas according to a uniform protocol described completely in another publication (*26*). Each animal was followed by semiweekly clinical examinations, repeat biopsies when indicated, and necropsy. Drugs were given in sequence beginning with prednisone, followed by cyclophosphamide, and later by vincristine. Biopsies repeated between each course of therapy were evaluated by cytochemical methods. Remissions were evaluated by measurement of nodes or other masses in 3 dimensions and by examination of peripheral blood and bone marrow. A satisfactory remission required at least 50% reduction in size of tumor within 14 days of beginning of therapy. Longevity was not always a valid criterion because owners sometimes requested euthanasia while animals were in remission. However, good response to therapy was often associated with significant prolongations of life. Many dogs which were presented in advanced stages of disease lived relatively normal and comfortable lives for 6–12 months after diagnoses, a significant period in the expected lifespan of a dog.

We observed distinct correlations between cytologic characteristics of neoplasms and response to chemotherapy, to the extent that we can predict which animals in the same clinical stages of disease will respond to drugs. Lymphomas consisting predominantly of histiocytes or blast cells with uniform, strong cytoplasmic pyroninophilia and low hydrolytic enzyme activity were resistant to prednisone and cyclophosphamide but were somewhat sensitive to vincristine. Those composed of cells with either some degree of nuclear chromatin condensation (prolymphocytes), weak cytoplasmic pyroninophilia, or higher enzyme activity were sensitive to all 3 drugs. Complete remissions were achieved in more than half the animals in the latter group.

Discussion

Despite the prolongations of human life achieved by use of antineoplastic drugs, effective chemotherapy is limited to a few types of neoplasms, and selection of agents is often empirical. The margins between efficacy and toxicity are narrow, and undesirable effects on non-neoplastic cells limit use of many drugs. Until drugs with more specific effects on tumor cells can be developed, opportunities for permanent cures will continue to be limited to a few types of neoplasms. Because the target of any therapy is the tumor cell, methods must be developed which will allow more accurate predictions of reactions of cells of individual neoplasms to drugs. Although we do not understand many of the basic biologic differences between "cancer" cells and "normal" cells, there is a need for rapid and reproducible clinical methods for evaluating biochemical differences in neoplasms. Cytochemical procedures performed on the actual tumor cells to be evaluated would have the advantage of speed and would take into account the individuality of neoplastic cells. Such methods could lead to development of more rational approaches to chemotherapy.

This study was designed to explore the possible use of certain rapid cytochemical methods as diagnostic procedures with the aim of improving accuracy of prognostication and prediction of response of individual lymphomas to chemotherapy. The cytologic and cytochemical procedures used in our preliminary studies are: 1) pattern of nuclear chromatin; 2) cytoplasmic pyroninophilia; and 3) hydrolytic enzyme activity.

Nuclear chromatin patterns indicate to some degree the stage of DNA replicative activity and may influence effects of drugs, particularly antimetabolites.

Ribonuclease-digestible pyroninophilia is an estimation of amounts of cytoplasmic RNA. The mode of action of prednisone on neoplastic lymphocytes is thought to be through enhancement of ribonuclease activity (*27*). Our observations, that strongly pyroninophilic cells are resistant to prednisone, might be explained by such a mechanism.

A possible explanation for the correlation of response to drugs with hydrolytic enzyme activity may be drawn from the work of Brandes and Anton (*28*) who demonstrated that lysosomes play a role in the cellular lytic process in mammary tumors treated with Cytoxan. The fact that high hydrolytic enzyme activity is associated with function of macrophages and corresponds to the "starry sky" phenomenon is an interesting observation because tumors which characteristically exhibit this feature, as the Burkitt tumor of African children (*29*) and the feline lymphomas, are extremely sensitive to chemotherapy.

Cytochemical studies of lymphomas of dogs appear to support concepts that biochemical activity can be correlated with the biological behavior of tumors and possibly with action of specific chemical agents. The degree of tumor cell differentiation correlates to some extent with course of disease in lymphomas of man (*30*). It may be possible to extend these correlations by development of more precise methods for predicting behavior of individual neoplasms.

Lymphomas of dogs are useful biomedical models for development of such methods.

Hodgkin's-Like Tumors

Hodgkin's disease is a human neoplastic syndrome with characteristic clinical and pathologic features. Until etiologic agents or processes are defined, a search for counterparts in animals must be based on morphologic or clinical similarities.

Neoplasms designated as Hodgkin's-like have been reported in animals of several species, most frequently in dogs (*31–36*). Most cases described in species other than dogs do not withstand critical evaluation. I have personally observed 4 examples of lymphomas of this type in dogs. All were widely disseminated sarcomatous tumors of lymph nodes, liver, spleen, and structures of the mediastinum which fulfilled histologic criteria for Hodgkin's disease including the presence of quite typical Reed-Sternberg cells. These presumed morphologic counterparts of Hodgkin's disease in dogs are rare, compared to incidence of lymphomas of other types.

Each of the 4 canine cases demonstrated effacement of nodal architecture by delicate-to-coarse areas of fibrosis and a pleomorphic cell population consisting of bizarre histiocytes and variable numbers of mature lymphocytes, plasma cells, and eosinophils. Histiocytes were large, their nuclei were vesiculate, nuclear membranes were coarse, and nucleoli were prominent. Each lobe or nucleus of binucleate or multinucleate cells, which fulfilled accepted criteria for Reed-Sternberg cells, contained a solitary, centrally located nucleolus, surrounded by a clear area (the so-called "bull's eye") connected to the nuclear membrane by delicate chromatin strands (figs. 14–16). Some binucleate forms were "mirror images." One dog exhibited tubercle-like granulomas and giant cells of the Langhans' type, in addition to several acceptable Reed-Sternberg cells.

Malignant lymphomas of cattle are similar to those of dogs and are predominantly acute sarcomatous diseases with variously recorded incidences of leukemia. Occasional bovine lymphomas contain bizarre histiocytic giant cells which fulfill some criteria for Reed-Sternberg cells. However, the underlying lymphomas do not have other characteristics of Hodgkin's disease, *i.e.*, fibrosis and inflammatory cell infiltrates. Because giant cells similar to Reed-Sternberg cells are occasionally observed in benign reactive lymphoid tissues of animals of several species, as in man, care must be exercised to distinguish benign from malignant processes.

The frequency of other types of lymphomas in animals, compared to the rarity of Hodgkin's types, is curious and suggests that etiologic agents or pathogenic mechanisms are relatively host-specific or that development of the disease requires a period that exceeds the lifespan of many animals. It is noteworthy that Hodgkin's disease rarely occurs in children.

We must continue to search for etiologic mechanisms for the many disseminated granulomatous diseases of animals for which etiologies are unknown. Although clinical and pathologic features of some suggest similarities to Hodgkin's disease, most do not satisfy all criteria. However, differences in pathologic manifestations of diseases in animals of different species do not necessarily preclude similar or identical etiologies.

Plasma Cell Tumors

Plasma cell tumors have been reported in animals of several species, most often in dogs (*37–41*). Precise incidences of diseases of this group in animals are not known, but rates are negligible compared to those of lymphosarcomas. The terminology used to describe neoplasms of plasma cells is confusing because some investigators consider the terms *myeloma* and *myelosarcoma* to indicate all neoplasms originating in bone marrow regardless of cell type, while others restrict use of the term *myeloma* to *plasma cell tumors* regardless of location.

A majority of reported plasmacytic neoplasms have been in mature male dogs. Most were solitary tumors originating in long bones of the limbs. Some showed involvement of vertebrae; others were plasmacytomas limited to soft tissues. Bence-Jones proteinuria was observed in one case (*41*) and several had hypergammaglobulinemia, although laboratory examinations for abnormal globulins were not performed in every instance.

In addition to neoplasms which are frankly plasma cell tumors, cells composing lymphocytic lymphomas may have features of plasma cells. In some it seems to be academic as to whether the tumors should be classified as disseminated plasmacytomas or lymphosarcomas. This overlapping of cytologic features of plasma cells and lymphocytes has been observed in human patients (*18*) giving rise to the term "plasmacytoid lymphocytes."

There are no reports in the literature describing therapy of plasma cell tumors of dogs. Our experience with therapy of canine lymphomas indicates that strongly pyroninophilic cells are relatively insensitive to the drugs used in our studies.

Histiocytoses

Disseminated histiocytoses are apparently rare in dogs, and we are not aware of reports of either lipid or nonlipid varieties. Through the courtesy of Col. F. M. Garner of the Armed Forces Institute of Pathology, I have had an opportunity to examine material from a 10-year-old male Irish setter in which a form of nonlipid histiocytosis was observed. The dog had presented with generalized lymphadenopathy and a mediastinal mass. The peripheral blood was not remarkable. At necropsy, lymph nodes of most groups, liver, spleen, and bone marrow were densely infiltrated by nodular and diffuse accumulations of oval or fusiform cells. The architecture of lymph nodes was completely effaced (fig. 17). Nuclei of cells which had many characteristics of histiocytes were delicate and neither chromatin nor nucleoli were conspicuous. Cytoplasm was eosinophilic, broad, and often branched. In imprints, the cells showed characteristics of fairly well-differentiated histiocytes with typical, finely stippled chromatin and delicate nuclear membranes. Nuclei were elongate or oval and were folded or reniform.

The proliferative process was limited to structures of the hematopoietic system and appeared to represent a variant of malignant histiocytosis as described by Rappaport (*18*).

MYELOPROLIFERATIVE DISEASES

Neoplasms originating in cells of bone marrow are very rare in domestic mammals as compared to their incidence in humans. There are isolated reports of granulocytic leukemia in dogs (*11*, *42*, *43*), and the incidence of this disease has been reported to account for about 8% (*11*) of hematopoietic neoplasms. In our experience, the incidence is approximately only 1% of all hematopoietic tumors. The leukemoid reaction associated with lymphosarcoma may be misleading and has accounted for a few erroneous reports in the early literature. It is surprising that myeloproliferative neoplasms are as rare in domestic animals as they appear to be. Radiation is known to be a factor in the origin of this type of leukemia, and animals are probably exposed to levels of background radiation similar to those of humans. Diagnostic or therapeutic radiation may be factors or, as speculated in the case of Hodgkin's disease, it may be that a longer induction period is required than in the case of virus-induced lymphomas, and that the shorter lifespan of dogs prevents its development.

The clinical and pathologic features of myeloproliferative leukemia of dogs are similar to acute myeloblastic or promyelocytic leukemia in man. However, there may be enormously high peripheral counts and severe hepatosplenomegaly which is more typical of the chronic diseases of man. Severe anemia, fever, apparent bone pain, and mild-to-moderate lymphadenopathy are other common symptoms.

As in man and other animals, most granulocytic leukemias of dogs involve neutrophilic precursors, although there are rare reports of eosinophilic and basophilic leukemias (*44*).

Other myeloproliferative disorders are observed less frequently in dogs than granulocytic leukemias. Erythremic myelosis has been reported (*45*), and I have seen one case of megakaryocytic myelosis in a 2-year-old male mongrel (fig.18). Apparently, neither erythroleukemia, polycythemia vera, or agnogenic myeloid metaplasia has been recognized.

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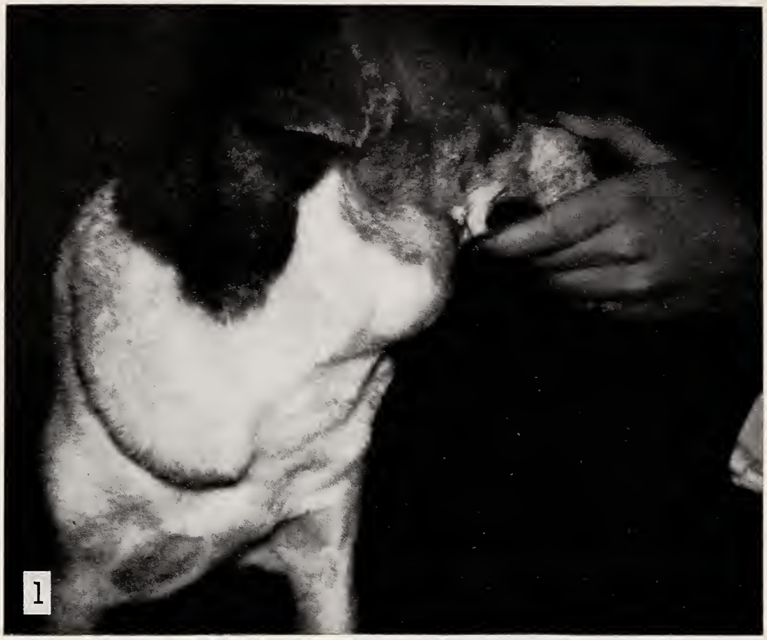
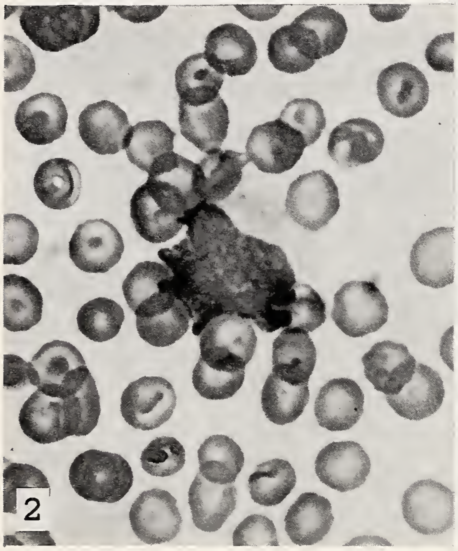




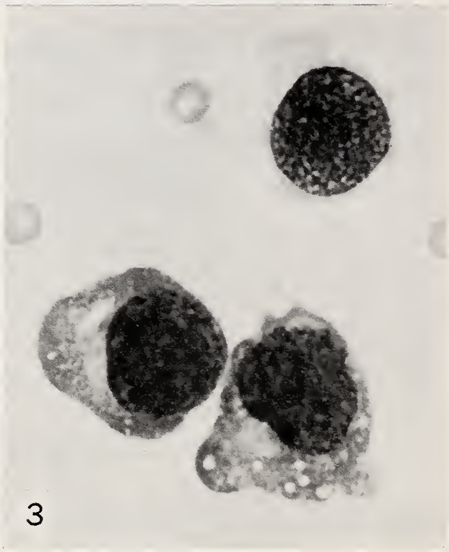
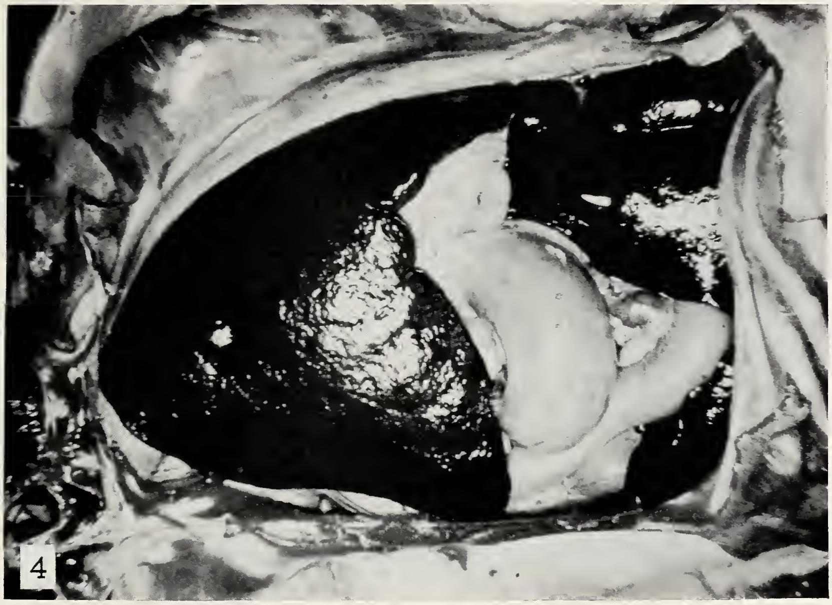


Figure 1.—Dog with lymphosarcoma involving submandibular and precapsular lymph nodes.

Figure 2.—Peripheral blood smear from dog with lymphosarcoma showing large, distorted lymphoblast. *Note* also several target cells. Giemsa. × 1100

Figure 3.—Peripheral blood smear from dog with lymphosarcoma showing circulating primitive stem cells. Giemsa. × 1100



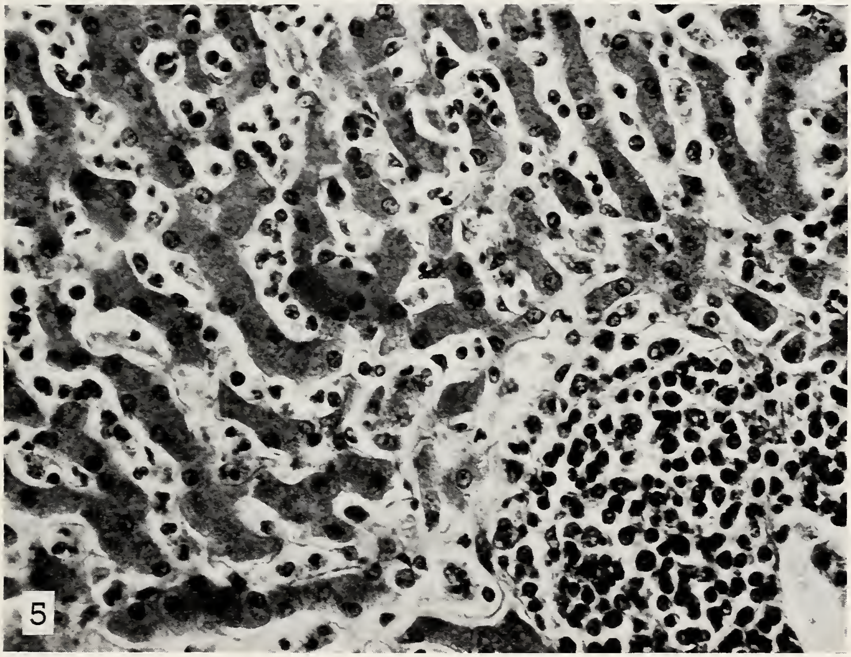
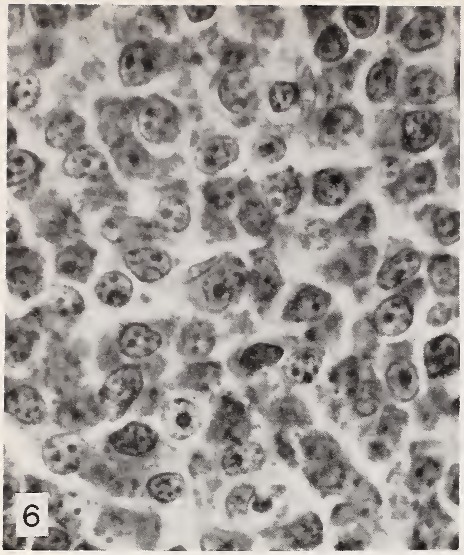
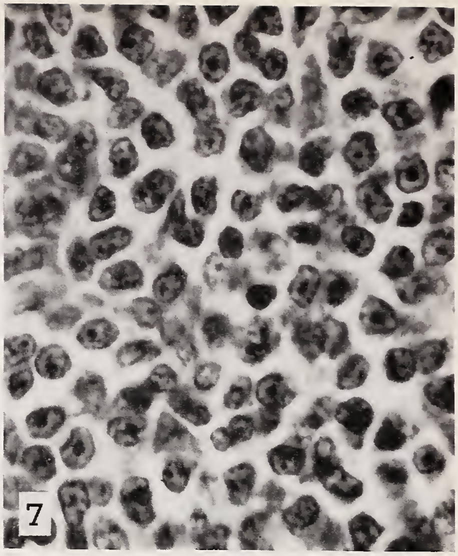


FIGURE 4.—Splenomegaly in dog with lymphosarcoma.

FIGURE 5.—Section of liver from dog with lymphosarcoma showing periportal and sinusoidal infiltrates. Hematoxylin and eosin. × 350





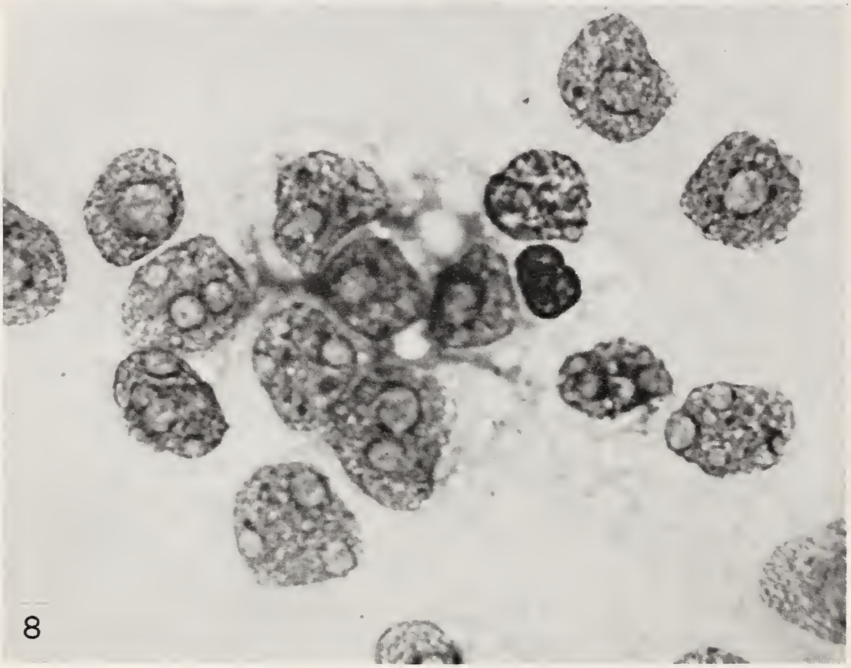
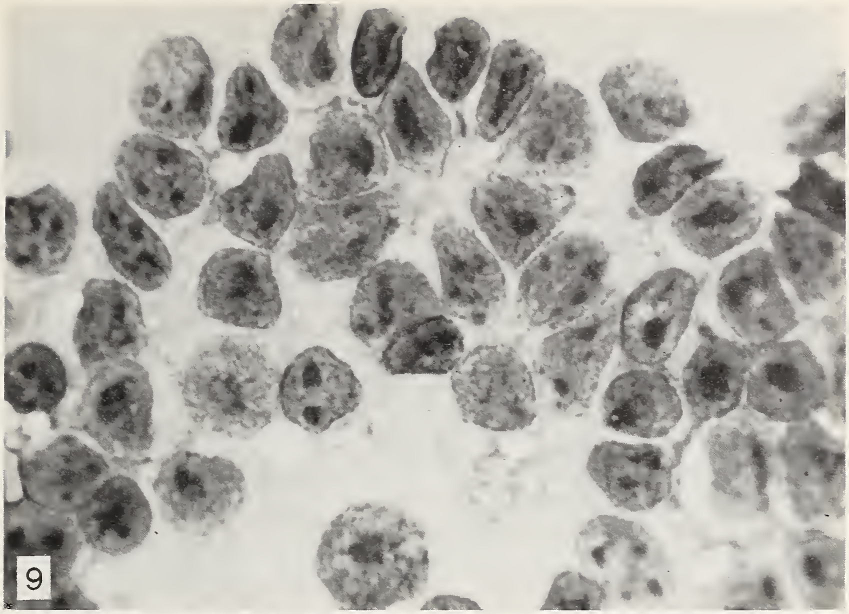


Figure 6.—Section of lymphosarcoma in dog. *Note* large vesiculate nuclei. This is the most common cytologic appearance in canine disease. Hematoxylin and eosin. × 1100

Figure 7.—Section of lymphosarcoma in dog showing smaller nuclei with coarser nuclear membranes. Hematoxylin and eosin. × 1100

Figure 8.—Fresh tumor imprint of canine lymphoma, histiocytic type. *Note* prominent nuclear chromatin stippling and huge nucleoli. Giemsa. × 1100



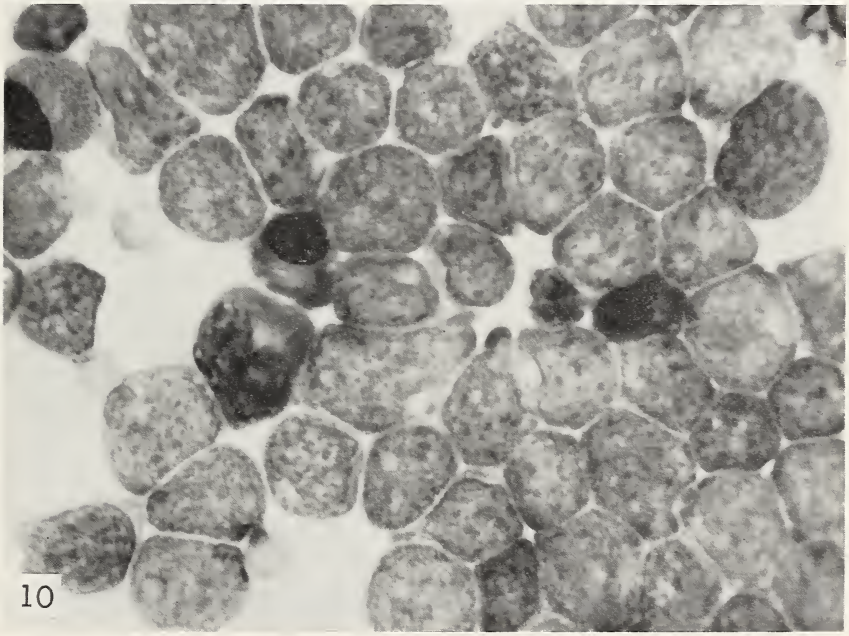
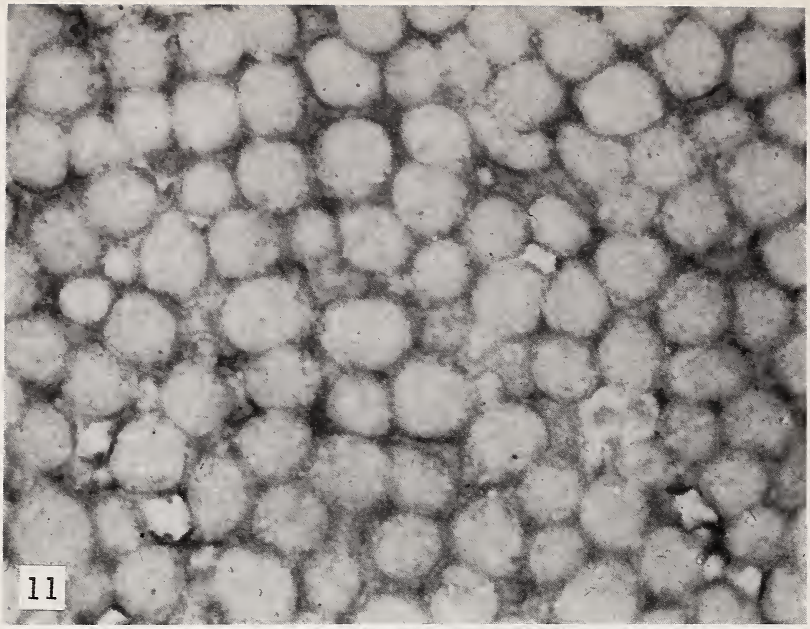


Figure 9.—Fresh tumor imprint of canine lymphoma, lymphoblastic type, showing finer, more uniform chromatin pattern. Giemsa. × 1100

Figure 10.—Fresh tumor imprint of canine lymphoma, prolymphocytic type. Chromatin particles are beginning to condense into larger aggregates. *Note* plasma cells, including one binucleate form. Giemsa. × 1100



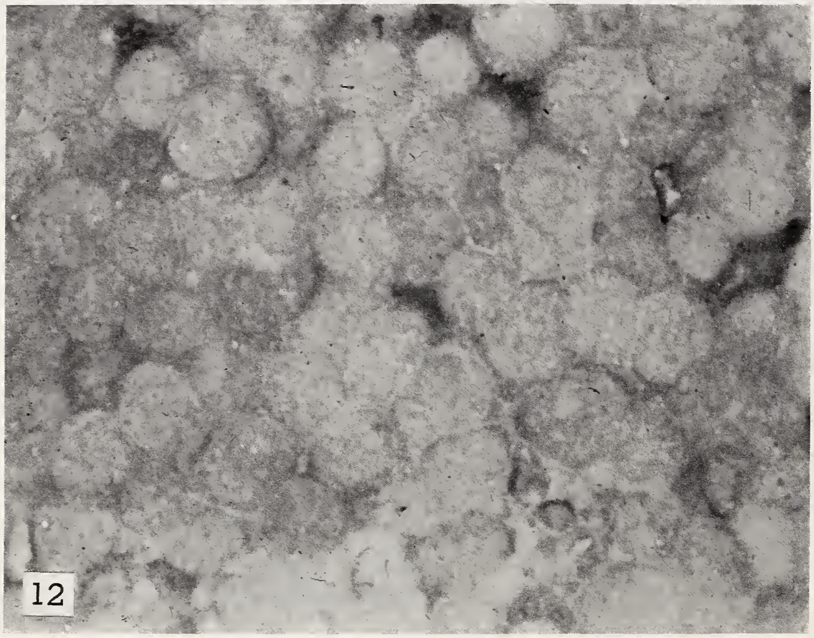
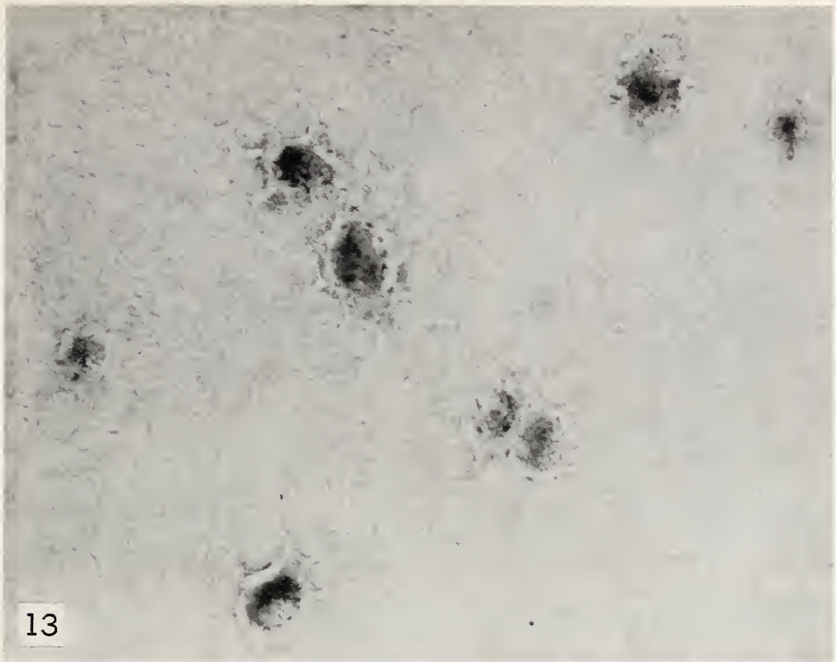
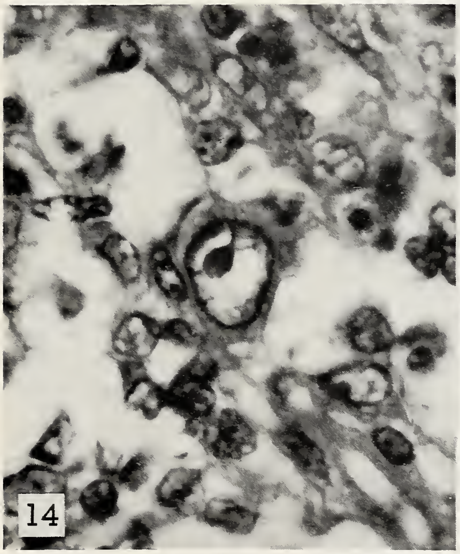


Figure 11.—Fresh tumor imprint of canine lymphoma, showing uniformly strong cytoplasmic pyroninophilia. Methyl green-pyronine. × 1100

Figure 12.—Fresh tumor imprint of canine lymphoma, showing weak cytoplasmic pyroninophilia. *Note* strongly pyroninophilic plasma cells compressed between tumor cells. Methy green-pyronine. × 1100





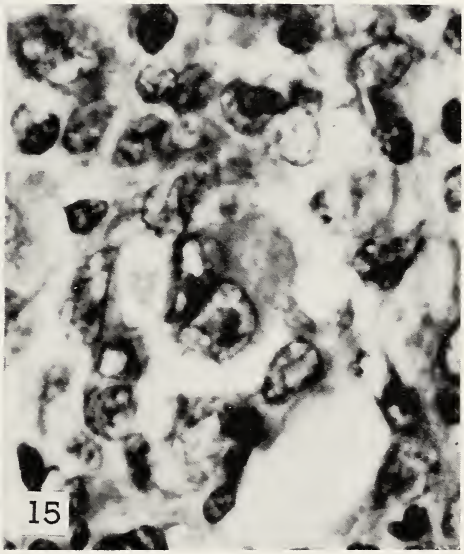
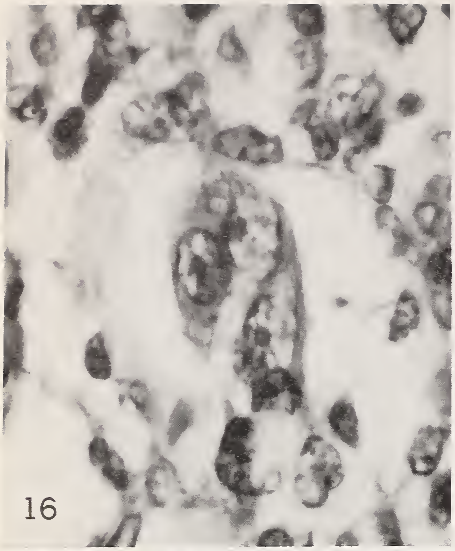
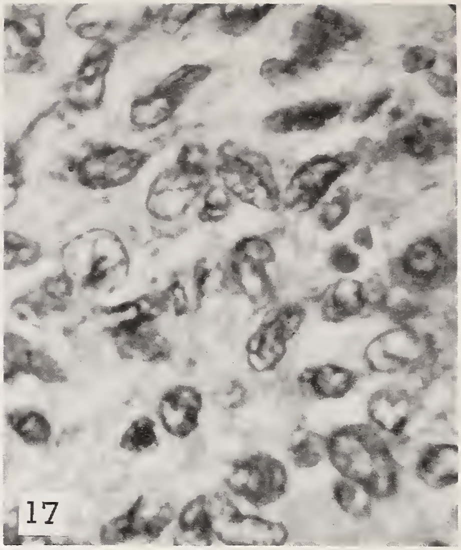


Figure 13.—Fresh unfixed tumor imprint, showing positive β-galactosidase activity in scattered cells. Indolyl substrate method; nuclear fast red counterstain. × 650

Figure 14.—Section of lymphoma, Hodgkin's-type, in dog, showing large "bull's-eye" reticulum cell. Pleomorphic cell population consists of reticulum cells, lymphocytes, plasma cells, and eosinophils. Hematoxylin and eosin. × 1100

Figure 15.—Same tumor as in figure 14. *Note* binucleate Reed-Sternberg cell. Hematoxylin and eosin. × 1100





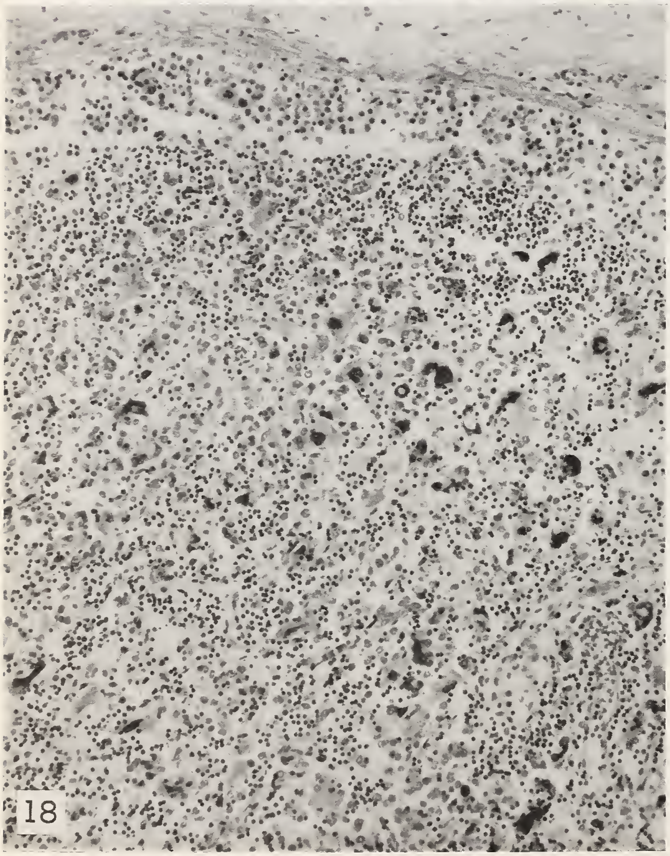


FIGURE 16.—Same tumor as seen in figure 14, showing a multinucleate Reed-Sternberg cell. Hematoxylin and eosin. × 1100

FIGURE 17.—Section of lymph node from dog with malignant histiocytosis. *Note* delicate, folded nuclei. Hematoxylin and eosin. × 1100

FIGURE 18.—Section of lymph node from dog with megakaryocytic myelosis. Bizarre megakaryocytes and their precursors have infiltrated the pulp and sinusoids. Hematoxylin and eosin. × 100

Chromosome Studies in Various Neoplasms of Domestic Animals [1]

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IN 1956, Tjio and Levan first described the presence of 46 chromosomes in human embryonic cells and thereby challenged the previous concept of 48 chromosomes as the normal number in humans. Subsequent studies in other human cells confirmed their observations and, in 1960, a standard system of nomenclature for chromosomes in mitosis was proposed at the Denver Human Genetics meetings. Chromosomes were classified into 3 types according to shape and location of the centromere: median (metacentric), submedian (submetacentric), and subterminal (acrocentric). These groups represent 22 pairs of autosomes and one pair of sex chromosomes.

The development of an improved technique made it possible to study the chromosomes of all living things including plants, fish, amphibians, birds, and other animals. For example, chickens have 9 pairs of large chromosomes and many minutes, the sex chromosomes being fifth in order, ZZ in male and ZW in female; the mouse has 40 acrocentric chromosomes; rats have 42 chromosomes; dogs have 78 chromosomes (76 acrocentric autosomes and 2 submetacentric sex chromosomes). Cats have 38 chromosomes, and elephants have 56 chromosomes.

Although chromosomes of many laboratory animal tumors have been studied, few studies have been performed on chromosomes of domestic animals. The only tumor for which chromosomes have been studied extensively is the canine transmissible venereal tumor (TVT) which is transplanted from one dog to another by coitus (*1*). The TVT can be transmitted by parenteral injection of viable tumor cells. The chromosome abnormalities found in two different studies [Makino, 1963, Japan (*2*), and Weber *et al.*, 1965, Philadelphia (*3*)] of the TVT deviated only slightly from each other in stemline patterns. All tumors had 59 chromosomes, in contrast to the normal canine number of 78 (table 1). Cases reported by Weber *et al.* contained 15 metacentrics or submetacentrics and 44

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Table 1.—Chromosome data on venereal tumors

Author	Number of dogs	Place	Chromosome data			
			Modal No.	Metacentrics or submetracentrics	Acrocentrics	Large negative heteropyknotic marker
Makino (*2*)	17	Japan	59	17	42	—
Weber *et al.* (*3*)	2	United States	59	15	44	+
Normal dog			78	0	78	0

acrocentrics, and both chromatids of the long arm of one large submetacentric showed markedly negative heteropyknosis. Cases reported by the Makino group contained 17 metacentrics or submetacentrics and 42 acrocentrics; no heteropyknosis was seen in any of the large submetacentric chromosomes.

The numbers of chromosomes in primary tumors in man or animals, whether or not induced by a virus, have rarely been constant from one individual to another. One exception is the Philadelphia (Ph^1) chromosome found in 85–90% of all patients with chronic myelogenous leukemia (CML). In human CML cases, the chromosome abnormalities deviated only slightly from the normal karyotype. However, if the TVT is a true primary neoplasm, it would be the first instance of such extensive, specific chromosomal changes in a neoplasm. Both Weber *et al.* and Makino suggested that the TVT may have originated in a single dog and has become a naturally transplanted tumor; whether a virus was involved initially is unknown.

Leukemia in dogs and cats is relatively rare compared to leukemia in humans. We have collaborated with Dr. Dean Buckner in the study of 5 dogs (table 2): 1 with acute lymphocytic leukemia (ALL), 1 with acute myelocytic leukemia (AML), 1 with chronic myelocytic leukemia (CML), and 2 with lymphomas. The only chromosomal aberrations were seen in the dog with AML which had an 81-chromosome cell line, both in the bone marrow and peripheral blood samples. No chromosomal abnormalities were seen in the dog with CML. Because all the autosomes in the dog are acrocentric, it was difficult to detect minor aberrations. No chromosomal abnormalities were noted in the dogs with lymphomas. In 1965, Basrur and Gilman (*4*) reported cytogenetic studies of 5 cases of lymphosarcoma in the dog. Three were found to have aberrated modal numbers, 77, 79, and 81, and the rest had normal modal numbers of 78. The altered stemline exhibited distinct chromosome features in each instance, although the alterations seemed to involve the addition of metacentrics with or without gain or loss of autosomes.

In three cases of lymphatic leukemia in cattle reported by Gustavsson and Rockborn (*5*) in 1964 in Sweden, the chromosome number was 59

Table 2.—Chromosome analysis of 5 dogs with neoplastic diseases

Diagnosis	Sample	Date	Chromosome distribution								
			76	78	79	80	81	82	83	Total	Hyperdiploid (%)
Lymphoma (case 1)	BM	12/27/66		50						50	0
	LN	12/27/66		50						50	0
Lymphoma (case 2)	BM	1/ 3/67		50						50	0
	LN	1/ 3/67		10						10	0
ALL	BM	9/12/66		50						50	0
	PB	9/12/66		50						50	0
CML	BM	1/15/66		50						50	0
AML (Akita dog)	BM	6/ 5/66	1	9	5	2	72	3		92	89
	PB	6/ 5/66					4			4	100
	BM	6/ 8/66		10		2	10	2	1	25	60

*BM = bone marrow; LN = lymph nodes; PB = peripheral blood.

rather than the normal number of 60. The animals had 3 submetacentric chromosomes instead of 2; the extra submetacentric chromosome was supposedly due to fusion or translocation of pair 1 and 29. The same chromosomal abnormality was found in a fetus from one of the cows. This finding may indicate that the 59 chromosomes were due to genetic rather than neoplastic changes.

Hare *et al.* (*6*) reported cytogenetic studies of 47 bovine lymphosarcomas; 34 had chromosome aberrations, namely, 1 hypodiploid, 25 hyperdiploid, and 8 tetraploid or near-tetraploid. In 10, one of the X chromosomes was missing and it may have been missing in 2 others.

More information concerning chromosomes of domestic animals will be required to evaluate the significance of chromosomal aberrations in available data.

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Hematopoietic Neoplasms of Slaughter Animals[1, 2]

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HEMATOPOIETIC NEOPLASMS observed in cattle, pigs, sheep, goats, and horses slaughtered in federally inspected extablishments of the United States are: *a*) *malignant lymphomas; b*) *thymomas; c*) *mastocytomas;* and *d*) *granulocytic sarcomas* (malignant myelomas).

Malignant lymphomas are the most frequently observed hematopoietic neoplasms of animals of all 5 species (tables 1 and 2). Rates in individual species cannot be compared to each other because of a lack of uniform information about such characteristics of slaughter animals as age, condition, and their care. Except for minor variations in distributions of neoplastic infiltrates, morphologic patterns are similar in all 5 species and resemble those seen in lymphomas of other animals and man.

TABLE 1.—Rates of occurrence of malignant lymphomas per 100,000 animals presented for slaughter, July 1957–June 1967

Fiscal year	Cattle	Calves	Sheep	Goats	Pigs	Horses and mules
1958	17. 63	1. 76	0. 37	0	1. 32	0
1959	18. 19	1. 91	. 61	0	1. 58	1. 14
1960	16. 92	2. 63	. 68	3. 28	1. 44	10. 45
1961	18. 86	2. 11	. 52	2. 34	1. 70	2. 02
1962	18. 79	2. 13	. 48	0	1. 90	0
1963	17. 06	1. 85	. 39	0	2. 07	10. 57
1964	17. 01	3. 10	. 41	1. 15	1. 84	0
1965	17. 91	2. 35	. 31	0. 77	1. 98	2. 89
1966	17. 89	2. 25	. 38	0. 57	1. 99	18. 75
1967	17. 51	2. 36	. 55	0. 99	1. 92	16. 54

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Table 2.—Histopathologically confirmed hematopoietic neoplasms submitted by veterinary meat inspectors, July 1957–June 1967

Neoplasm	Cattle	Calves	Sheep	Goats	Pigs	Horses and mules
Malignant lymphomas	900	9	17	2	200	3
Chronic granulomatous disease	—	—	—	—	35	—
Thymomas	29	—	2	12	5	2
Mastocytomas	12	—	—	—	5	—
Granulocytic sarcomas	2	—	—	—	2	—
Plasma cell neoplasms	—	—	—	—	—	—

Thymomas occur in animals of all 5 species (table 2). They are encapsulated thoracic masses—composed of mixtures of reticular and lymphocytic cells—characteristically benign and rarely metastatic. Goats appear to have a relatively high incidence of thymomas compared to animals of the other 4 species.

Mastocytomas, rare in slaughter animals were found only in cattle and pigs (table 2).

Granulocytic sarcomas (malignant myelomas), also rare in slaughter animals, were observed only in cattle and pigs (table 2). Distribution of infiltrates was characteristic of granulocytic sarcoma rather than of leukemia. The outstanding characteristic was the green color of gross organs.

MATERIALS

Material from more than 18,000 animals was histopathologically examined in the Pathology Laboratory of the Federal Meat Inspection Service of the U.S. Department of Agriculture during the 10-year period 1957–67. Descriptions of hematopoietic neoplasms in this report are based on examination of material submitted by veterinary meat inspectors (table 2). In federally inspected slaughterhouses, antemortem and postmortem examinations of all animals are performed by uniform systematic procedures (*1*). If a veterinary meat inspector detects clinical signs and/or lesions at necropsy that are unusual or atypical or that require histopathologic confirmation, he submits representative material and a written description of gross observations to the Pathology Laboratory.

Calves are defined as cattle younger than 1 year. Cattle, pigs, and sheep can be divided into two groups with potentially important implications in the epizootiology of neoplasms and comparative oncology. One group, *feeders,* are young, sexually mature animals (cattle 1–3 years of age, pigs 6–8 months, and sheep under 1 year) that have been confined in feedlots for feeding of concentrated fattening rations for a specific number of days before slaughter. The other group, *adults,* are older animals kept for varying lengths of time for breeding and other commercial purposes and sent to slaughter when they become unproductive. Approximately 80% of cattle, 92% of pigs, and 93% of sheep slaughtered under federal meat inspection specifications are feeders and the remainder are adults. Most goats and horses are adults when slaughtered.

Occurrence rates of hematopoietic neoplasms other than malignant lymphomas cannot be determined accurately because veterinary meat inspectors submit material for histopathologic confirmation only when gross lesions are unusual or questionable. Obvious neoplasms are not always submitted for confirmation. The laboratory receives disproportionately high numbers of relatively unusual tumors.

Because submitted specimens may be limited to material from primary sites, extent of disease cannot always be determined. Descriptions based on postmortem examinations are often limited to the most extensive lesions. Information about involvement of other structures is often minimal. In contrast to animals included in epizootiologic surveys or hospital clinic populations, most animals slaughtered for food are young, just sexually mature, and in a well-nourished state. These factors must be considered when data on distribution and characterization of neoplasms observed in slaughtered animals are compared to those from sources in which diseases are permitted to run their course.

Studies of material from animals condemned in slaughterhouses offer opportunities to collect material from relatively large populations of animals. They have their disadvantages in that complete clinical and epidemiological information is usually not available.

Numbers of animals slaughtered in the United States each year in establishments under federal jurisdiction are recorded in table 3 (*2–5*). These figures represent approximately 80% of the animals slaughtered in the United States.

TABLE 3.—Numbers of animals slaughtered in U.S. federally inspected establishments, July 1957–June 1967

Fiscal year	Millions					
	Species					
	Cattle	Calves	Sheep	Goats	Pigs	Horses and mules
1958	18.58	6.65	12.88	0.17	59.20	0.13
1959	17.32	5.14	12.80	.19	63.87	.09
1960	18.46	4.98	13.45	.06	70.50	.07
1961	19.86	5.20	14.92	.09	64.21	.05
1962	20.16	5.01	14.67	.10	67.11	.05
1963	20.86	4.77	14.14	.26	69.31	.05
1964	23.20	4.54	13.70	.17	72.16	.04
1965	25.81	5.02	12.32	.26	68.54	.03
1966	27.38	4.92	11.65	.35	60.66	.04
1967	27.87	4.19	11.63	.50	68.68	.04

HEMATOPOIETIC NEOPLASMS

All material submitted to the laboratory by veterinary meat inspectors is fixed in 10% buffered formalin. Except for occasional use of special stains such as Giemsa, Wilder's reticulum, and toluidine blue, paraffin-embedded sections are stained routinely with hematoxylin and eosin.

MALIGNANT LYMPHOMAS

Malignant lymphomas are also called lymphosarcoma, leukosis, lymphoblastoma, lymphadenosis, lymphomatosis, lymphocytoma, aleukemic leukemia, and pseudoleukemia.

Surveys of neoplasms of farm animals from several countries of the world indicate that lymphomas are among the most prevalent neoplasms of large domestic animals (*6–13*). Such surveys indicate not only relative frequencies of neoplasms of different cell types in a single species but also provide some information concerning relative frequencies of specific neoplasms in more than one species.

A review of malignant lymphomas in domesticated mammals has been provided by Jarrett *et al.* (*14*) who described etiologic and epidemiologic studies in Europe and in the United States. The review by Smith (*8*) summarized knowledge of malignant lymphomas in animals, particularly cattle. Bendixen's report (*16*) of bovine malignant lymphomas (leukosis) in Denmark illustrates the complexity of this disease in its various manifestations. Several investigators (*17–21*) have reported epizootiologic studies of bovine malignant lymphomas in specific geographic areas of the United States. We are not aware of similar studies of malignant lymphomas in slaughter animals other than cattle.

The only statistics on incidence of malignant lymphomas of large domestic animals other than cattle in the United States are those of the Federal Meat Inspection Service (*2–4*) (table 1). Actual rates are probably higher than those recorded because these statistics do not include animals that died on farms or that were slaughtered in establishments not under the jurisdiction of federal meat inspectors.

During a 10-year period (July 1957 through June 1967), rates of malignant lymphomas of cattle, pigs, and sheep remained relatively constant (table 1). Average rates, expressed as numbers of lymphomas observed per 100,000 slaughtered animals per year, were: cattle, 18/100,000; calves, 2/100,000; pigs, 2/100,000; and sheep, 0.5/100,000. The apparent differences in annual rates of lymphomas of goats, horses, and mules reflect variations in numbers, ages, and conditions of animals slaughtered in a given year and cannot be interpreted as actual differences in incidences.

Gross Descriptions

Malignant lymphomas are usually white to grayish-white, fleshy masses with little or no lobulation. Color depends on the amounts of necrosis, hemorrhage, and congestion. Malignant lymphomas may be nodular or diffuse. Consistency depends on amounts of supporting connective tissue. Malignant lymphomas involve many organs and tissue structures, as described by Smith (*22*) in his study of malignant lymphoma in 1,000 slaughtered cattle.

Classification of Malignant Lymphomas

The term *malignant lymphoma,* defined by Gall and Mallory (*23*) and later by Gall and Rappaport (*24*), has been used by the U.S. Federal Meat Inspection Service since 1953 (*25*) to designate various forms of lymphoid neoplasms.

Malignant lymphomas observed in slaughter animals can be classified into four major types. An additional category designated *granulomatous* includes those rare animal neoplasms which resemble Hodgkin's disease of man. Cell types vary morphologically; the various categories overlap.

a) The *lymphocytic type* is characterized by cells that are uniform in size and shape. Staining density varies little. Component cells are slightly larger than normal lymphocytes. Nuclei are round and chromatin granules tend to be concentrated near nuclear membranes. Cytoplasm is scant (fig. 1).

b) The *lymphoblastic type* is composed of cells slightly larger than those of the lymphocytic type. The cells are of about the same size and shape. Nuclei are large and round. Abundant chromatin granules, evenly distributed within the nuclei, look like hyperchromasia. Some nuclei may be indented. Cytoplasm is more abundant than in cells of the *lymphocytic type,* and cytoplasmic boundaries are distinct.

c) The *histiocytic type* is characterized by cellular pleomorphism. Variations in cells in a single field are more obvious at high magnifications. Component cells are larger and vary more in size and shape than those of the *lymphoblastic type.* Most nuclei are oval and may be indented or kidney-shaped. Pale eosinophilic cytoplasm is abundant and cytoplasmic processes may extend in various directions (figs. 2 and 3). Bengston (*26*) in 1938 first reported a *reticulum cell sarcoma* in a cow. He used silver stains to demonstrate the "reticular" nature of histiocytes or "reticulum cells." Small, spherical "punched-out" areas ("starry sky") are sometimes observed in the histiocytic type, particularly near necrotic areas (fig. 4).

d) The *mixed types* are malignant lymphomas composed of cells of two or more cell types.

e) The *granulomatous type* is characterized by fibrosis and proliferation of histiocytes and polymorphonuclear eosinophils. Occasional multinucleated giant cells may be observed. Hemorrhage and congestion of involved organs are prominent. The *chronic granulomatous disease* of pigs, described by Forbus and Davis (*27*), is included in this category. Similar processes have not been described in other slaughter animals except for a report by

Runnells and Benbrook (*28*) who described a malignant lymphoma of a horse in which microscopic lesions resembled those of Hodgkin's disease.

As in man, reactive hyperplasias of animal lymph nodes are often difficult to distinguish from neoplastic processes. Criteria for distinguishing reactive and neoplastic changes in lymph nodes have been described by Rappaport (*29*). Because regional lymph nodes are included in most routine specimens received in our laboratory, regardless of the primary disease, we have had many opportunities to observe the various histomorphologic changes occurring in nodes which drain sites subjected to a variety of insults; changes in many of these nodes are proliferative and may mimic lymphomas. The problem is less difficult when there is complete obliteration of nodal architecture by a lymphomatous process or when a neoplasm can be demonstrated in other organs or tissues of the body.

Malignant Lymphomas of Cattle

Of material submitted to the Pathology Laboratory by veterinary meat inspectors during 1957–67, diagnoses of malignant lymphoma were confirmed by histologic examination in 900 cattle and 9 calves (table 2). Malignant lymphoid neoplasms in very young cattle have been reported by Hatziolos (*30*), who described a malignant lymphoma in a bovine fetus, and by Misdorp (*31*), who observed lymphatic leukoses in 2 newborn calves. Eighty-five percent of cattle of our series in which diagnoses of lymphomas were made were 3 years old or more. Approximately 20% showed evidence of poor health at slaughter, although no definite correlations could be made between cell types of lymphomas and effects on general health of animals. Clinical signs and courses of disease would of course depend on anatomic sites of involvement, duration, and extent of disease. Clinical features of 59 bovine lymphomas in which the disease was allowed to run its course have been described by Marshak *et al.* (*17*).

Lymph nodes were involved in most bovine lymphomas of our series. Evaluations of enlarged lymph nodes of cattle, like those of other animals, depend on several factors and details of clinical histories, and postmortem examinations must be considered in addition to the histologic appearance of the node. Because lymph nodes of calves normally contain active lymphoid tissue, special care must be taken when the nodes are evaluated. Lymphomatous lymph nodes from cattle may be enlarged as much as 4–6 times. They are whitish gray and friable. Petechiae and focal areas of necrosis may be observed.

The heart was involved in more than 50% of cattle (fig. 5). The malignant process is believed to begin in the right auricle. The neoplastic cells characteristically infiltrate the myocardium, gradually replacing the muscle fibers (fig. 6). Jarplid (*32*) explained the high incidence of right auricular involvement in cattle on the basis that subepicardial tissue remains undifferentiated and responds readily to proliferative stimuli.

The stroma of the myocardium and heart valves are apparently the last structures to be destroyed by the malignant process, since hydrothorax and passive congestion of the liver were rare in these animals.

Spleens were affected in approximately 19% of cattle. They were enlarged as much as 3–5 times. The cut surface was maroon and of semisolid consistency, in contrast to the bright-red and bloody appearance of a congested spleen.

Hemal nodes, histologically and physiologically similar to the spleen, are found only in ruminants (*33*). They are located near the hilus of lymph nodes in various organs and in subcutaneous adipose tissue—especially on the back and in other sites. Hemal nodes may be involved in lymphomas. Because they are easily accessible for biopsy, some investigators have suggested biopsy of subcutaneous hemal nodes as a method for early diagnosis of malignant lymphoma (*34*).

Kidneys were involved in approximately 18% of cattle. The neoplasm was usually nodular and multiple. Nodules were most numerous in cortices with growth toward medullary regions (fig. 7). These infiltrates must be differentiated from those of a more common non-neoplastic inflammatory process, focal nonsuppurative embolic nephritis. In lymphomas, walls of ureters and urinary bladder may also be invaded: In these organs, neoplastic cells proliferate in lamina propria and invade other layers.

The uterus was involved in at least 10% of bovine lymphomas. This is probably a low estimate because the uterus, being an "inedible" organ, is not ordinarily examined so thoroughly as other organs. Despite widespread involvement of other organs by a malignant lymphoma, the uterus may not be examined, or if examined, may not be specifically described in necropsy reports. Lymphomas of the uterus usually appear as multiple, large, nodular masses in the wall (figs. 8 and 9). In advanced stages, the uterine wall may be diffusely thickened, up to 8 cm.

Livers were involved in approximately 10% of adult cattle and in most calves of our series. Hepatic foci were characteristically multiple, whitish-gray nodules of varying sizes distributed throughout the parenchyma. Some livers, which did not contain grossly visible nodules, were enlarged and pale, an appearance characteristic of diffuse infiltrations by neoplastic cells. Microscopically, several variations in patterns of neoplastic involvement of livers were noted. In some, neoplastic cells were arranged in discrete nodular masses. In others, the cells formed wide collars around hepatic lobules. In a few, neoplastic lymphoid cells were found only in the sinusoids (fig. 10) despite lack of evidence of leukemia in other organs. Because hematologic information was not available for slaughter animals, we could not correlate morphologic patterns with hematologic information.

The walls of the gastrointestinal tract, particularly of the abomasum (fourth or glandular compartment of stomach of ruminants), were involved in 8% of animals. A higher incidence of gastrointestinal involvement might have been anticipated because of the presence of lymphoid tissue in the walls.

HEMATOPOIETIC NEOPLASMS

Lungs were involved in 9% of animals. The infiltrates were distinct, whitish nodules which ranged from 2–4 cm in diameter. These pulmonary infiltrates probably originated in intrapulmonary lymph nodules.

In fewer than 1% of cattle, malignant lymphomas involved the vertebral canal in the region of the terminal spinal cord (fig. 11). Clinically, involvement of the spinal canal is known to paralyze hind limbs by pressure on motor nerves.

Multiple, ovoid masses of tumor measuring up to 30 cm in diameter were observed in thoracic, abdominal, and pelvic cavities of 16% of cattle. These masses of tumor probably originated in lymph nodes. Clinically, such masses in pelvic regions are known to cause paresis of hind limbs by pressure on nerve trunks.

Lymphomatous nodules of the skin were observed in fewer than 1% of animals. All animals with skin infiltrates also had involvement of lymph nodes. Marshak *et al.* (*35*) described in detail a generalized malignant lymphoma with cutaneous involvement in a heifer, and Carson and Jones (*36*) described one in an 8-year-old cow. Clegg and Moss (*37*) described skin leukosis in a 3-year-old heifer which died 3 years later from malignant lymphoma of the heart. Bendixen (*16*) believes that skin involvement indicates a distinctive type of malignant process with a pathogenesis different from that of other forms of bovine lymphoma (leukoses).

Thymic involvement was observed in lymphomas of at least 2% of cattle in our series and was always a part of a generalized process that included lymph nodes and other organs. It was frequently difficult to determine whether a mass in the anterior thorax was thymus, or included thymus. We have recorded thymic involvement only in those animals in which the organ was specifically identified at necropsy or in those in which Hassall's corpuscles or other characteristic structures were observed in histologic sections. Dungworth *et al.* (*38*) reported details of 14 generalized malignant lymphomas of cattle in which thymus was said to be the organ most severely affected.

True thymomas are quite different from malignant lymphomas and are described in a separate section of this report.

Exophthalmus, associated with malignant lymphoma of the orbit and retrobulbar tissues, was observed in several cattle.

We did not have an opportunity to examine bone marrow from any cattle. However, we know that bone marrow involvement occurs in significant numbers of malignant lymphomas of cattle, as described by Dungworth *et al.* (*38*).

The relationship between leukemia and malignant lymphoma has not been established. Weber (*39*) reported that only 5–10% of cattle affected by malignant lymphoma had hematologic evidence of leukemia. Herzog (*40*) and Thompson and Roderick (*41*) also reported little correlation between hematologic findings and malignant lymphoma. In Denmark, much emphasis is placed on the number of lymphocytes in the peripheral blood, and persistent lymphocytoses in adults are considered to indicate a pretumorous stage. Those interested in this theory should consult the work of Bendixen (*16*).

The extent of involvement by malignant lymphoma in slaughtered cattle is difficult to determine because not all affected organs or body systems are mentioned specifically in necropsy reports. However, in most cattle the lymphomas were specifically described in more than one group of lymph nodes and/or body systems. Malignant lymphoma may occur in almost any part of the body as either a primary or a metastatic neoplasm.

Morphologic classification of bovine lymphomas of 909 cattle of our series indicated that 20% were *lymphocytic,* 38% *lymphoblastic,* 35% *histiocytic,* and 7% *mixed.* Granulomatous forms were not observed in cattle. Morphologic characteristics of cells varied in different affected nodes and organs in specific animals.

Malignant Lymphomas of Pigs

Lymphomas are observed in slaughtered pigs at an average annual rate of 2/100,000 (tables 1 and 2). Sixty percent of 200 histologically confirmed cases in our series were in pigs 6–8 months old. It must be emphasized that 92% of all pigs slaughtered are this age, and valid comparisons of rates between species cannot be made.

Twenty-five percent of affected pigs showed some clinical evidence of disease possibly related to underlying lymphomas. In more than 90% of pigs, lymphomas involved more than one body system or group of lymph nodes, although the disease did not appear to be as widespread in the body as in cattle. In several animals, lymphomas were found only in the liver (figs. 12 and 13) and/or kidney. Lungs and gastrointestinal tract were less often involved. In contrast to lymphomas of cattle, those of pigs rarely involved the heart. Infiltrates were not observed in uterus or spinal meninges.

Microscopic examination revealed that approximately 42% of porcine lymphomas were *lymphocytic,* 34% were *lymphoblastic,* 15% *histiocytic,* and 9% *mixed.* Tumor cell distributions within affected organs were similar to those of cattle.

Chronic Granulomatous Disease of Pigs

During the 10-year period, we had an opportunity to study material from 35 pigs with the chronic granulomatous disease described in 1946 by Forbus and Davis (*27*) (table 2). This disease is described in this paper because the original investigators believed that morphologic changes resembled those of human Hodgkin's disease.

Incidence of this granulomatous disease of unknown etiology is probably higher than our figures indicate because veterinary meat inspectors who submit material for histologic confirmation are usually not familiar with the disease.

Seventy percent of pigs with this entity were 1 year of age and older, which suggests that the disease is one of older animals, although the process may begin relatively early in life. Characteristics of the clinical course are not known because the disease had not been recognized during life; evidence of anemia and icterus was observed in some carcasses, however. The disease has never been linked to an infectious agent.

Grossly, the most striking and constant necropsy findings were the greatly enlarged and often massive and hard spleens (from 4–15 times normal size) (figs. 15 and 16), which contained many red-and-white mottled areas, and enlarged cirrhotic livers. One spleen weighed 17 pounds; the normal pig's spleen weighs ½ to 1 pound on the average. Livers were enlarged 2–4 times and were pale because of excessive fibrosis. Thick, white interlobular connective tissue bands separated hepatic lobules. Lymph nodes, kidneys, and bone marrow were sometimes affected. Lymph nodes were enlarged and congested when involved in the malignant process.

Microscopically, much normal splenic parenchyma had been replaced by a diffuse granulomatous process. Early stages were characterized by infiltrations of histiocytes and eosinophils, congestion, and hemorrhage. Advanced stages were characterized by diffuse fibrosis and depletion of lymphoid tissue (fig. 17). The differences in degrees of cellular infiltrates and fibrosis suggest that the disease is chronic and that we observed different stages of the disease process. Multinucleated giant cells, some of which resembled megakaryocytes, were observed in spleens of some animals (fig. 18). Cells with features of Reed-Sternberg cells were not observed in animals of our series although they have been reported (*27*).

A diffuse granulomatous process with extensive proliferation of interlobular connective tissue was noted in livers. Small collections of polymorphonuclear eosinophils and mononuclear leukocytes were scattered in connective tissue (fig. 19). Proliferation of biliary ducts was observed in livers of some animals. Gradual encroachment on hepatic lobules resulted in atrophy or destruction of liver cells in advanced stages of the disease.

In most pigs the lymph nodes were not affected, although in nodes of some animals, a granulomatous process similar to that of the spleen was observed (fig. 20).

Forbus and Davis reported that focal granulomatous lesions similar to those of the spleen were also observed in bone marrow and kidneys.

The etiology and pathogenesis of this disease need clarification.

Malignant Lymphomas of Sheep

Malignant lymphomas of sheep occur at a rate of approximately 0.5/100,000 slaughtered animals per year. Of 17 histologically confirmed lymphomas of sheep of our series, all but 2 were in older animals (table 2). All sheep had multiple neoplasms in different parts of the carcasses distributed like cattle lymphomas. The lymph nodes were always affected, and generally were involved in more than one site in a single animal. Liver, spleen, and kidneys were involved in 40% of the cases. The diaphragm was infiltrated in 3 animals, the subcutis of the skin in 2 (fig. 21), lungs in 2, the abomasum in 1, and peritoneum in 1. Heart, uterus, and thymus were not involved in any animals.

Microscopically, 10 ovine lymphomas were *lymphocytic* and 7 were *histiocytic*. The histomorphologic changes in affected organs resembled those in affected organs of cattle.

Malignant Lymphomas of Goats

The numbers of goats slaughtered for food is comparatively small, and annual rates vary compared to those of other animals; therefore, the annual rates of malignant lymphomas also vary (tables 1 and 3). Of the two histologically confirmed lymphomas of goats (table 2), one in a 4-year-old female slaughtered in poor condition had involvement of many skeletal and mesenteric lymph nodes. The other, in a 3-year-old female slaughtered in good condition, had involvement of all skeletal and visceral lymph nodes, heart, liver, and lungs.

Microscopically, the lymphoma of the former goat was of the *lymphocytic* type and that of the latter was of the *histiocytic* type. Histomorphologic changes observed in caprine lymphomas were similar to those of cattle.

Malignant Lymphomas of Horses and Mules

Many reports of malignant lymphomas in horses have appeared in the literature (*28, 42, 43*). Cornelius *et al.* (*44*) described in 1959 a plasma cell myelomatosis in a 16-year-old gelding (castrated male). The number of horses slaughtered for food in the United States has decreased gradually during the past 10 years (table 3). The apparent variations in rates of occurrence of malignant lymphoma in horses of our series (table 1) may be explained in part by irregular culling practices; slaughter populations are not uniform from year to year.

Two of the three histologically confirmed equine lymphomas (table 2) were in horses and one was in a mule. Both horses were aged. In one horse, all body lymph nodes were involved. In the other horse, only lumbar and sacral lymph nodes, muscles of the diaphragm, and extensor muscles of the rear legs were involved. The mule, aged and in poor condition, had cervical, mediastinal, and lumbar lymph nodes enlarged 20–25 times normal.

Microscopically, the lymphomas of both horses were classified as *histiocytic;* the lymphoma of the mule was *lymphocytic.*

THYMOMAS

Thymomas were observed in animals of all 5 species. In our experience they are relatively benign and are composed of cells of both reticular (epithelial) and lymphocytic types. Usually encapsulated, they do not ordinarily metastasize, though they may invade thoracic viscera.

In a few tumors one or the other of the two cell types may predominate, or the neoplasm may be composed entirely of cells of one type. When lymphocytic cells predominate, thymomas are sometimes difficult to distinguish from lymphomas of the thymus. Castleman (*45*) described criteria for diagnosis of thymoma. As already stated, thymomas are usually encapsulated and rarely metastasize, whereas malignant lymphomas usually involve other organs in addition to thymus. Sections should be taken from several sites of thoracic tumors suspected of being thymomas, because ratios of component cells can vary in different areas of a tumor.

Thymomas of Cattle

Jackson (*46*) described in detail 4 thymomas of cattle. Twenty-nine bovine thymomas were examined in our laboratory (table 2). Twenty-four of these occurred in cattle over 3 years old.

Grossly, the typical bovine thymoma was a nodular mass ranging from 10–20 cm in diameter. It was usually located in the anterior thorax (fig. 22). Some thymomas, however, were located in the lower neck anterior to the thoracic inlet. In one animal the neoplasm entered the thoracic inlet from the lower neck region. Most thymomas were encapsulated and lobulated. Color varied from grayish-white to yellow to light brown. Focal areas of hemorrhage and necrosis were occasionally observed on cut surfaces. The tumor was often attached to the lung, pericardium, or parietal pleura. Depending on the size and location of the neoplasm, varying degrees of interference with the functions of the structure of the anterior thorax and thoracic inlet may occur. Growth apparently progresses by expansion rather than by infiltration; metastases were observed in mediastinal lymph nodes of only one animal.

Microscopically, bovine thymomas typically had well-defined connective tissue capsules which in some areas were quite thick. Wide connective tissue septa extending inward from the capsule divided tumors into lobules of varying sizes. Frequently, neoplastic cells invaded the septa. Delicate stroma supported neoplastic cells. Hemorrhages, focal areas of necrosis, and infiltration by polymorphonuclear neutrophils were occasionally noted. Hassall's corpuscles or their degenerated forms were observed in 3% of tumors; this percentage would probably have been higher if serial sections had been examined. Two definite neoplastic cell types were recognized in bovine thymomas: *a*) a small, round cell morphologically resembling a lymphocyte and *b*) a larger cell with a vesicular oval nucleus, a prominent nucleolus, and relatively abundant cytoplasm which resembled a reticulum cell. Probably both types of cells always occur in "typical" thymomas, but this is sometimes difficult to demonstrate if one type predominates.

In 20 bovine thymomas, the predominant cells were reticular cells, arranged in broad sheets. In these, small collections of lymphocytes were scattered thoughout. Reticulum cells palisaded around small blood vessels in several cases (fig. 23). In the other 9 thymomas, lymphocytes predominated, with significantly fewer reticulum cells scattered thoughout.

Thymomas of Pigs

Thymomas are rare in slaughtered pigs. All 5 histologically confirmed thymomas (table 2) occurred in 6-month-old pigs. All were located in the anterior thorax and measured 8–15 cm in diameter. They were grayish white with focal areas of necrosis. There were distinct capsules. No metastases were noted.

Microscopically, thymomas of pigs were similar to those of cattle. Reticulum cells predominated. Interestingly, "starry sky" effects (phagocytic macrophages) were observed in a few thymomas and were most numerous near areas of necrosis.

Thymomas of Sheep

Thymomas are apparently less common in sheep than in other animals. We found only 2 in our series (table 2). Both thymomas were located in the anterior thorax of adult female sheep. One, a firm encapsulated mass 12 cm in greatest dimension, was attached to the apical lobe of the right lung. The other, an encapsulated tumor, measured 30 cm and was not attached to other viscera. Neither metastasized. Microscopically, thymomas of sheep were similar to those of cattle. The predominant cell was the lymphocyte.

Thymomas of Goats

The rates of occurrence of thymomas in goats appear to be higher than those in other species of slaughter animals. During a short-term survey of neoplasms of goats in 1958 by the Federal Meat Inspection Service, 8 thymomas were discovered in approximately 100,000 slaughtered goats. Of 12 goats with histologically confirmed thymomas recorded during a 10-year period (table 2), 10 were 2–6 years old and 2 were 1 year old. All goats were in poor or fair condition before slaughter.

Grossly, the thymomas were large, tan, encapsulated masses located in the anterior thorax. They were firm and lobulated, ranging from 5–15 cm. One tumor weighed 8 pounds. Some tumors had focal areas of necrosis. Some were attached to lungs and had caused extensive subpleural atelectasis by their expansive growth. No tumors metastasized.

Microscopically, the thymomas were covered by thick, dense connective tissue capsules (fig. 24), and thick septa extended from capsules into the tumors. Morphologic characteristics of caprine thymomas were similar to those of bovine thymomas (fig. 25). Lymphocytes predominated in all tumors. Hassall's corpuscles were demonstrated in more than half the tumors.

Thymomas of Horses

Thymomas are apparently rare in horses. Both equine thymomas recorded in table 2 were in aged horses. One was a 15 cm encapsulated, cream-colored mass attached to the parietal pleura. The other was a 4 × 9 cm lobulated mass adjacent to the trachea in the lower neck. Neither tumor metastasized.

Microscopically, in both horses, reticulum cells predominated; small collections of lymphocytes were scattered throughout (fig. 26).

MASTOCYTOMAS

Mastocytomas are also called mast cell tumors, basophilic myelosarcomas, and mastosarcomas. Mastocytomas are composed of cells resembling and presumably derived from tissue mast cells. Cytoplasm characteristically contains prominent granules that stain metachromatically with certain aniline dyes. Although tissue mast cells have little morphologic resemblance to blood basophils, the biochemical components of the two cell types are similar, and the mastocytoma has also been called a basophilic myelosarcoma.

Mastocytomas were observed only in cattle and pigs of our series.

Mastocytomas of Cattle

In contrast to dogs, in which mastocytomas, particularly of the skin, occur frequently, cattle rarely have mastocytic neoplasms, according to a review by Groth *et al.* (*47*) who reported a mastocytoma in a bovine abomasum. Dodd (*48*) described a similar tumor in the tongue of a calf.

Mastocytomas are rare in slaughtered cattle. Only 12 bovine mastocytomas were confirmed in our laboratory (table 2). Ten cattle were adults; 2 were younger than 3 years.

Grossly, bovine mastocytomas were nodular masses ranging from 1–7.5 cm in greatest dimensions. They varied from white to yellow to green, depending on the amount of necrosis and number of polymorphonuclear eosinophils present. Some had foci of calcification. The mastocytomas were multiple in all cattle except one in which the mast cell infiltrates were in only one lymph node. Characteristically, bovine mastocytomas involve lymph nodes, skin, liver, spleen, lungs, skeletal muscles, heart, kidneys, omentum, pleura, pericardium, and peritoneum.

Microscopically, bovine mastocytomas were nonencapsulated, discrete nodular masses (fig. 27). Cells were clustered and were supported by delicate connective tissue stroma. In some tumors, they were separated into distinct lobules by prominent connective tissue septa. The neoplastic mast cells were similar to those of mastocytomas of other animals. Nuclei were eccentrically located, but occasionally they were centrally located. Coarse, discrete chromatin granules were prominent (fig. 28). A few nuclei were indented. Abundant eosinophilic cytoplasm and distinct cell walls were characteristic in fixed sections stained with hematoxylin and eosin. Staining with aniline dyes, such as Giemsa, revealed metachromatic cytoplasmic granules. Polymorphonuclear eosinophils observed in mastocytomas varied in number. Most eosinophils were found near blood vessels in connective tissue septa.

Mastocytomas of Pigs

Mastocytomas are relatively uncommon in pigs. Only 5 were observed in pigs of our series (table 2). All were located in dermis of the skin. Animals ranged in age from 6 months to 1½ years. The mastocytomas were nodular, measured 0.5–2.5 cm in diameter, and were a definite pink. They were observed in skin over entire carcasses. In fact, in one animal, they were even observed in the dermis of the head, ears, and feet. Mastocytomas are easily observed in slaughtered pigs because the epidermis is routinely removed in the dehairing process; they would be difficult to observe in intact animals. No metastatic lesions were observed in any carcasses.

Microscopically, the mastocytomas were discrete, circumscribed nodules located in the dermis. Cells were separated into nests of variable sizes by relatively thick connective tissue septa that branched into more delicate thin septa separating smaller groupings of cells (fig. 29). The large number of septa is characteristic of this neoplasm. As in mastocytomas of cattle, polymorphonuclear eosinophils in the tumors varied in amount from area to area but were concentrated mostly adjacent to septa. Neoplastic mast cells were round-to-polyhedral, with abundant cytoplasm and well-defined cell walls, and tended to be arranged in sheets. Nuclei were round and eccentrically located and contained distinct chromatin granules. Metachromatic granules were difficult to demonstrate.

Mastocytomas of Other Animals

Mastocytomas were not observed in sheep, goats, or horses.

GRANULOCYTIC SARCOMAS

Granulocytic sarcomas are also called malignant myeloma, myelosarcoma, chloroma, myelogenous leukemia, and myeloblastic sarcoma.

Granulocytic sarcomas were observed only in cattle and pigs (table 2). Typically, they are focal or diffuse proliferations of immature granulocytes (myelocytes or myeloblasts) and have granules that may be eosinophilic, neutrophilic, or basophilic.

Neoplasms composed of immature granulocytes are also called malignant myelomas or myelosarcomas because of the *myeloid* origin of neoplastic cells. In human hematopathology, the term "myeloma" is used to designate neoplasms composed of plasma cells, so-called "multiple myeloma." Immature granulocytes are quite different from plasma cells, and confusion of the names is unfortunate.

Granulocytic Sarcomas of Cattle

Granulocytic sarcomas are rare in cattle, and only a few have been reported in the literature (*49*, *50*). Several examples were described in our earlier survey (*12*). All granulocytic sarcomas were composed of eosinophils; we did not see neutrophilic or basophilic types.

Two examples of granulocytic sarcomas of cattle examined in our laboratory are recorded in table 2. One was in a 20-month-old heifer and the other in a 6-year-old cow. Grossly, the outstanding characteristic was a green color observed in lymph nodes, liver, spleen, kidneys, lungs, and bone marrow. Affected organs were 2–3 times their normal size. The green color of gross organs was similar to that described in granulocytic sarcomas or chloromas of man (*29*).

Microscopically, neoplastic granulocytes formed discrete masses in distributions similar to those observed in some malignant lymphomas. Delicate stroma supported neoplastic granulocytes. Nuclei were round or ovoid and hyperchromatic and were located slightly eccentrically (fig. 30). Some nuclei were slightly indented. Cytoplasm contained numerous distinct eosinophilic granules. Most neoplastic granulocytes were similar to, but larger than, normal eosinophilic myelocytes or myeloblasts. Mature polymorphonuclear eosinophils were scarce.

In the liver, neoplastic granulocytes were observed in interlobular connective tissue and had formed wide collars around hepatic lobules, similar to infiltrates of some malignant lymphomas. Although hematologic information was not available, there was no evidence of leukemia; neoplastic granulocytes were not observed in sinusoids or blood vessels.

Eosinophilic granulocytic sarcomas must be distinguished from two nonneoplastic diseases of cattle in which a gross green color is associated with infiltrations of many mature eosinophils: *a*) *eosinophilic lymphadenitis*, in which the gross green color is associated with accumulations of mature eosinophilic leukocytes in sinuses of lymph nodes and *b*) *eosinophilic myositis* (*51*), in which appear massive accumulations of mature polymorphonuclear eosinophilic leukocytes in striated muscles.

Granulocytic Sarcomas of Pigs

Granulocytic sarcomas are apparently rare in pigs, as in cattle. Both histologically confirmed examples of granulocytic sarcomas in pigs of our series were in adult females (table 2). The color of the gross neoplasms was green like that of cattle. Visceral and skeletal lymph nodes, liver, heart, kidneys, bone, pancreas, and serous membranes were involved. In microscopic sections, infiltrates were composed of large cells resembling eosinophilic myeloblasts and myelocytes, except that they were larger (figs. 31 and 32). Few mature eosinophilic leukocytes were present.

Granulocytic Sarcomas of Other Animals

Granulocytic sarcomas were not observed in sheep, goats, or horses.

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Figure 1.—Malignant lymphoma (lymphocytic type), lymph node, bovine. *Note* uniformity of cells and scant amount of cytoplasm. Hematoxylin and eosin. Armed Forces Institute of Pathology (AFIP) negative 68-6492-1. × 500

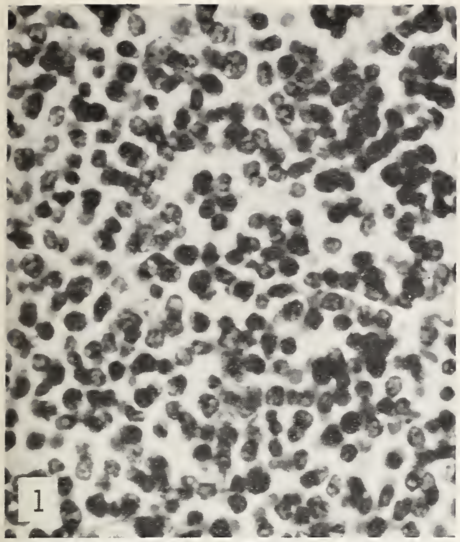
Figure 2.—Malignant lymphoma (histiocytic type), lymph node, bovine. Cells are large and have much cytoplasm and a vesicular nucleus containing a prominent nucleolus. Hematoxylin and eosin. AFIP negative 68-6492-2. × 600

Figure 3.—Malignant lymphoma (histiocytic type), lymph node, goat. *Note* large vesicular indented nuclei with prominent nucleoli. Hematoxylin and eosin. AFIP negative 68-6492-3. × 500

Figure 4.—Malignant lymphoma, lymph node, pig, showing "starry sky" effect. *Note* phagocytized nuclear debris. Hematoxylin and eosin. AFIP negative 68-6492-4. × 220

Figure 5.—Malignant lymphoma, heart, bovine. *Note* multiple nodular growths at base of heart. AFIP negative 68-6492-5.

Figure 6.—Malignant lymphoma, heart, bovine, showing diffuse infiltration of neoplastic cells between muscle bundles and muscle fibers. Hematoxylin and eosin. AFIP negative 68-6492-6. × 40



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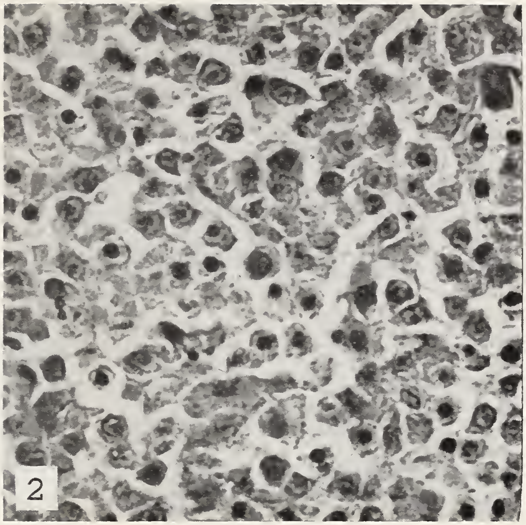

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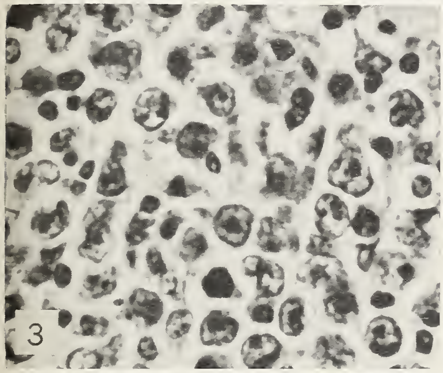

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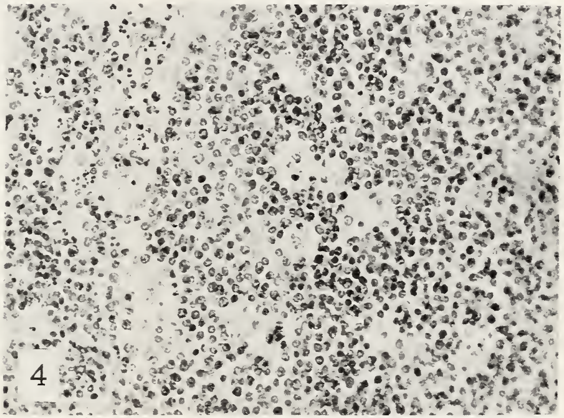

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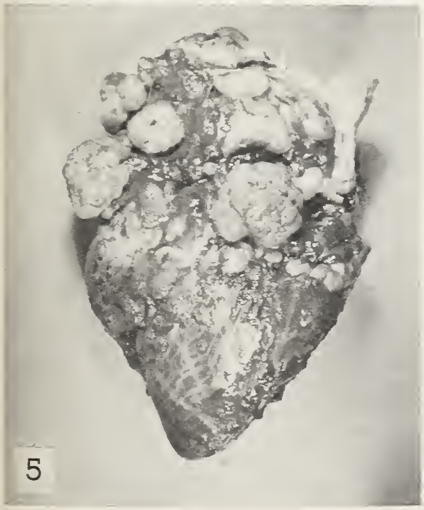

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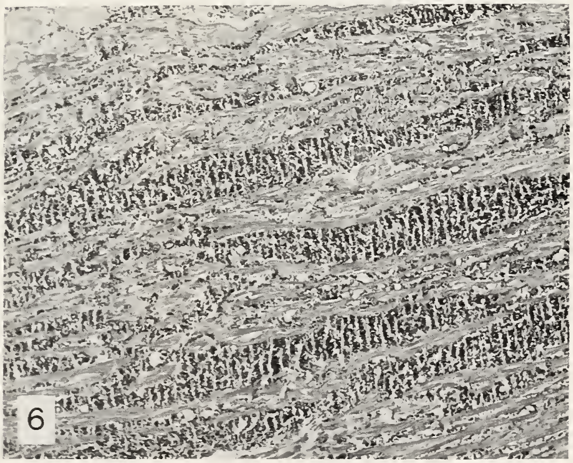

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Figure 7.—Malignant lymphoma, kidney, bovine, showing multiple nodular neoplastic masses. Armed Forces Institute of Pathology (AFIP) negative 68-6492-7.

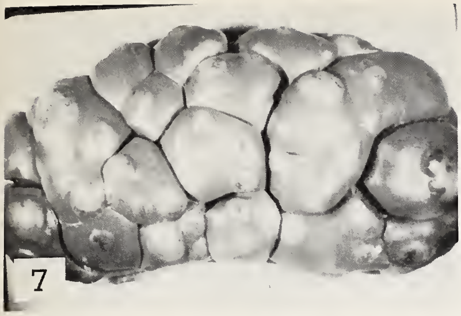
Figure 8.—Malignant lymphoma, uterus and cervix, bovine, showing multiple nodular growths on mucosal surface. AFIP negative 68-6492-8.

Figure 9.—Malignant lymphoma, uterus, bovine, showing infiltrations of neoplastic cells in endometrium and myometrium. Ductal structures in *center* of field are uterine glands. Hematoxylin and eosin. AFIP negative 68-6492-9. × 35

Figure 10.—Malignant lymphoma, liver, bovine, showing neoplastic cells in sinusoids and central vein. Hematoxylin and eosin. AFIP negative 68-6492-10. × 150

Figure 11.—Spinal meninges, bovine, showing nodular growths of malignant lymphoma. AFIP negative 68-6492-11.

Figure 12.—Malignant lymphoma, liver, pig, showing multiple nodular growths. AFIP negative 68-6492-12.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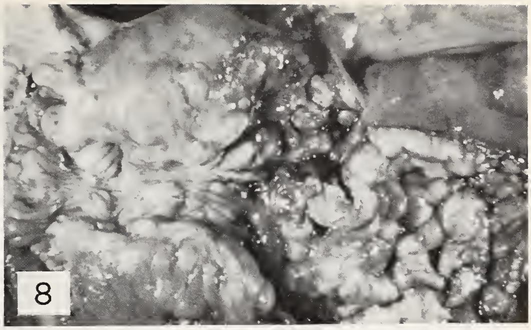
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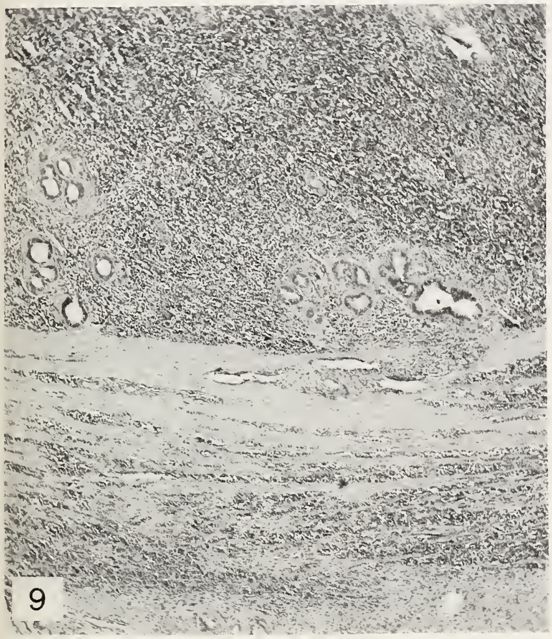
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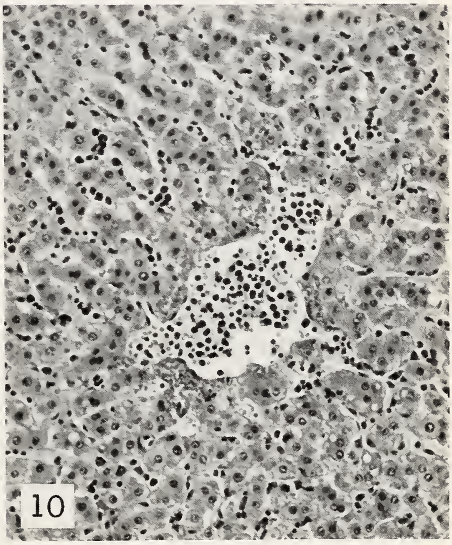
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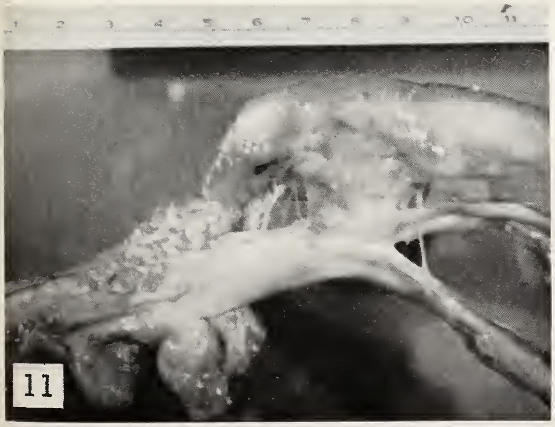
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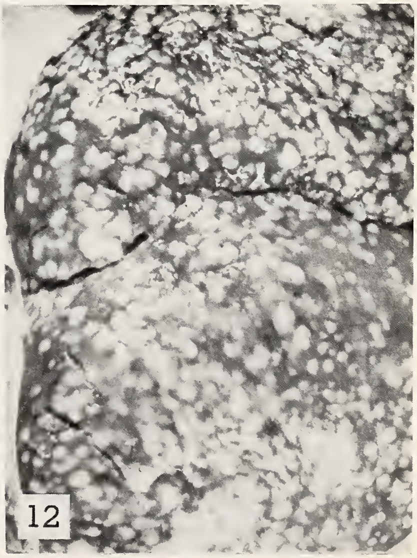
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Figure 13.—Malignant lymphoma, liver, pig, showing extensive infiltration of neoplastic cells which have almost completely replaced cells of liver cords. Hematoxylin and eosin. Armed Forces Institute of Pathology (AFIP) negative 68-6492-13. × 70

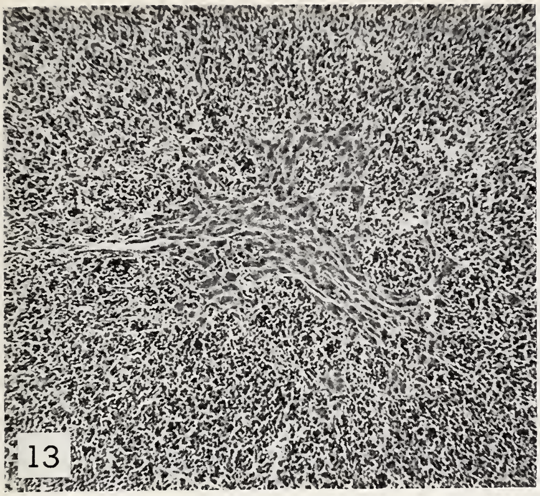
Figure 14.—Malignant lymphoma, thymus, pig, showing infiltration by neoplastic cells. Large concentric structures are remaining Hassall's corpuscles. Hematoxylin and eosin. AFIP negative 68-6492-14. × 130

Figure 15.—Chronic granulomatous disease, spleen, pig, showing enlarged spleen. For comparison, normal spleen is shown (*nearest ruler*). AFIP negative 68-6492-15.

Figure 16.—Close-up view of whitish-gray mottled spleen of pig with chronic granulomatous disease. AFIP negative 68-6492-16.

Figure 17.—Chronic granulomatous disease, spleen, pig. *Note* extensive fibrosis and scarring of white pulp. Hematoxylin and eosin. AFIP negative 68-6492-17. × 35

Figure 18.—Chronic granulomatous disease, spleen, pig, showing giant cells. Hematoxylin and eosin. AFIP negative 68-6492-18. × 500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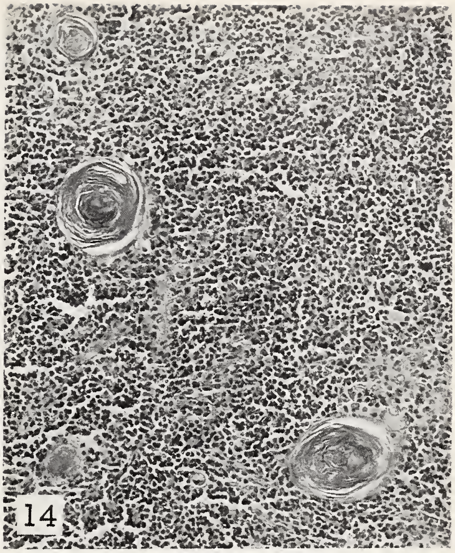
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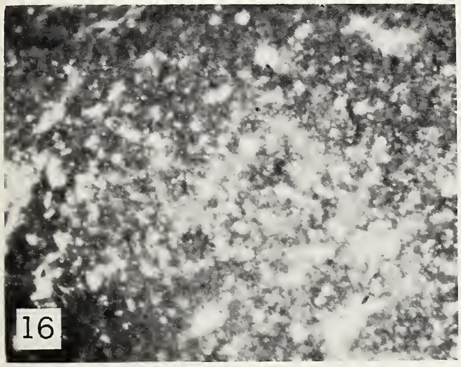
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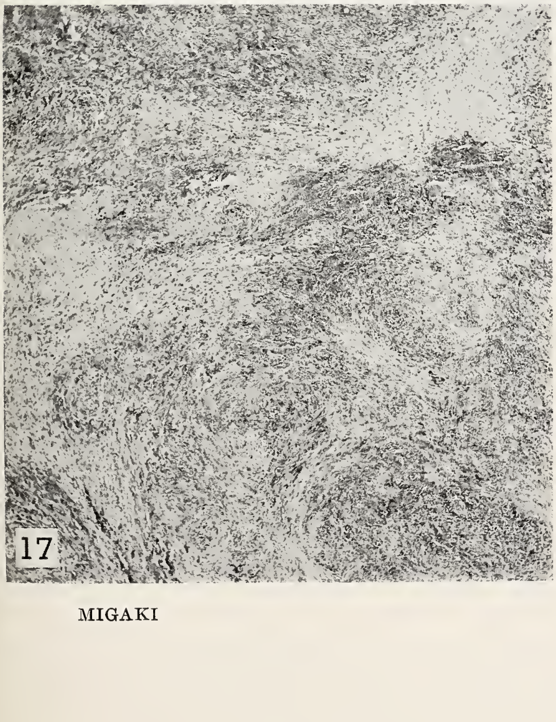
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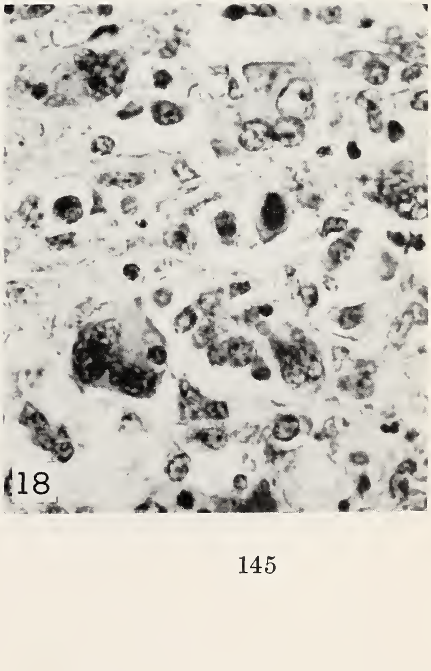
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FIGURE 19.—Chronic granulomatous disease, liver, pig. *Note* large amount of interlobular connective tissue containing small, diffuse collections of leukocytes. Hematoxylin and eosin. Armed Forces Institute of Pathology (AFIP) negative 68-6492-19. × 35

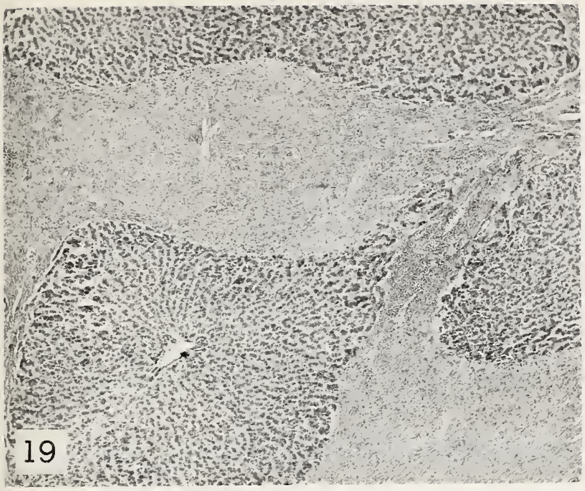
FIGURE 20.—Chronic granulomatous disease, lymph node, pig, showing extensive fibrosis of germinal centers. Hematoxylin and eosin. AFIP negatives 68-6492-20. × 115

FIGURE 21.—Malignant lymphoma, subcutaneous tissue, sheep. *Note* tumor cells invading dermis. Lymph nodes are also affected. Hematoxylin and eosin. AFIP negative 68-6492-21. × 70

FIGURE 22.—Thymoma, bovine, showing encapsulation and lobulation. AFIP negative 68-6492-22.

FIGURE 23.—Thymoma, bovine. *Note* presence of many reticulum cells with fewer smaller, dark-staining lymphocytes. Reticulum cells palisade around a small blood vessel. Hematoxylin and eosin. AFIP negative 68-6492-23. × 400

FIGURE 24.—Thymoma, goat. *Note* thick capsule and septa. Marked differences in staining quality of neoplastic cells are seen at this magnification. Hematoxylin and eosin. AFIP negative 68-6492-24. × 115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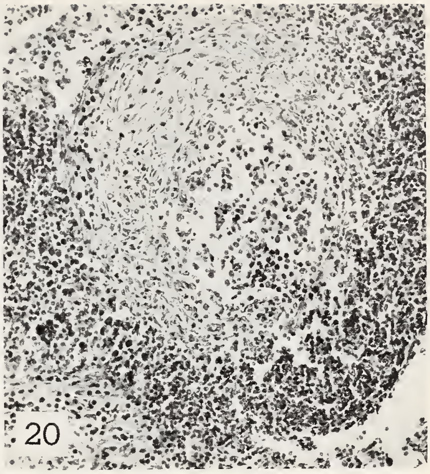
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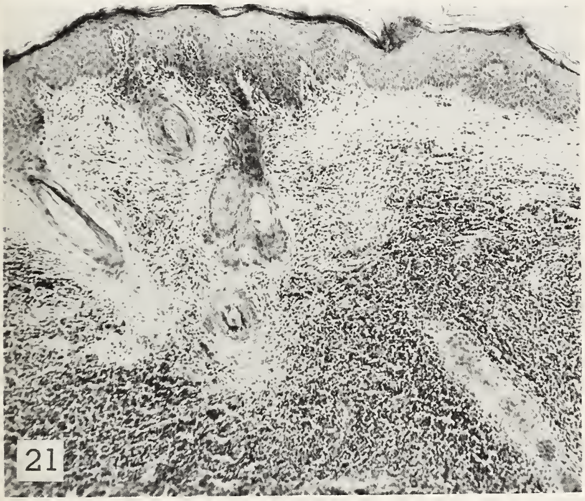
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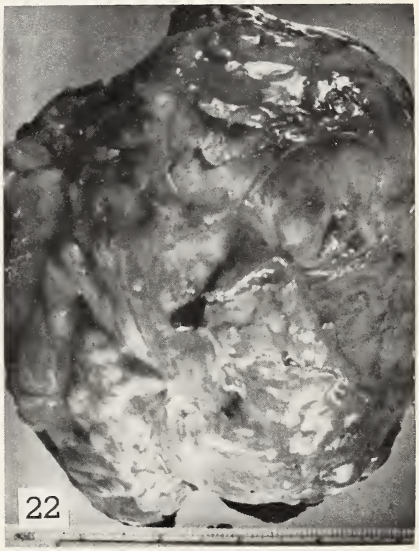
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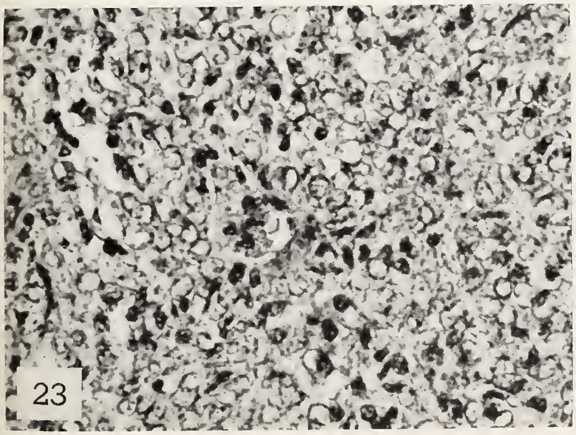
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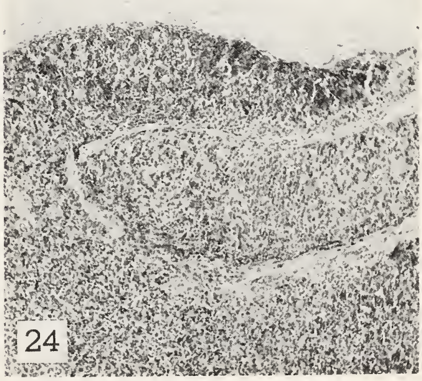
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Figure 25.—Thymoma, goat. *Note* lobule formation by thick connective tissue septa. Hematoxylin and eosin. Armed Forces Institute of Pathology (AFIP) negative 68-6492-25. × 80

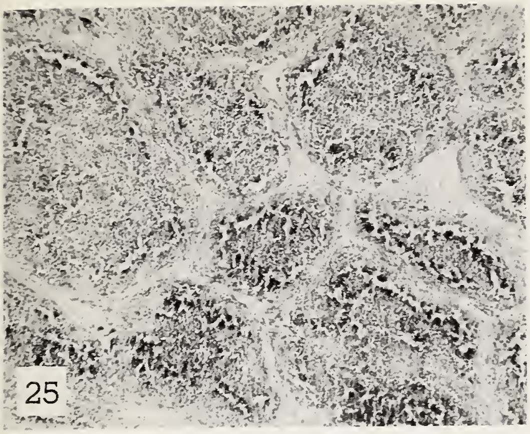
Figure 26.—Thymoma, horse, showing two cell types. Larger, light-staining cells are reticulum cells, and dark-staining cells are lymphocytes. Hematoxylin and eosin. AFIP negative 68-6492-26. × 500

Figure 27.—Mastocytoma, liver, bovine, showing nodular appearance. Growth occurs by expansion rather than infiltration. Hematoxylin and eosin. AFIP negative 68-6492-27. × 100

Figure 28.—Mastocytoma, lung, bovine. *Note* neoplastic cells lining thin connective tissue septa. Dark-staining cytoplasmic granules are in these cells. Smaller cells are polymorphonuclear eosinophils. Giemsa. AFIP negative 68-6492-28. × 600

Figure 29.—Mastocytoma, dermis of skin, pig. Neoplastic cells are separated into lobules by connective tissue septa. Glandular structures beneath the neoplasm are sweat glands. Hematoxylin and eosin. AFIP negative 68-6492-29. × 40

Figure 30.—Granulocytic sarcoma (malignant myeloma), liver, bovine. Dark-staining ovoid nuclei are eccentrically located, and a moderate amount of cytoplasm can be seen. *Note* delicate stromal tissue supporting neoplastic cells. Giemsa-stained sections showing eosinophilic granules in cytoplasm. AFIP negative 68-6492-30. × 530



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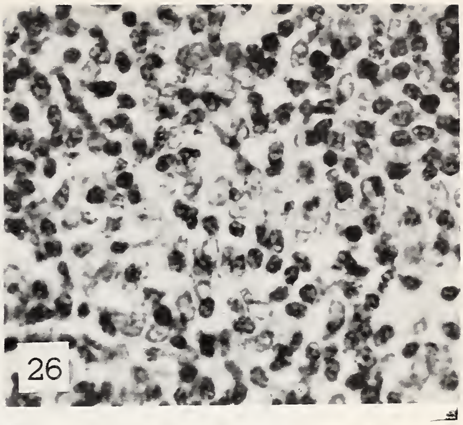

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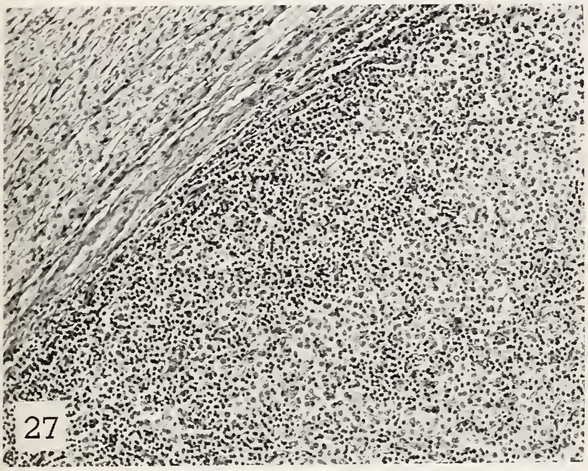

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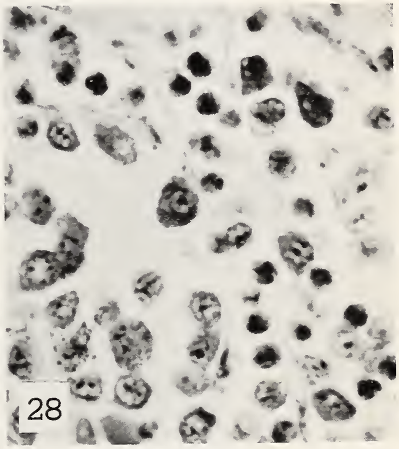

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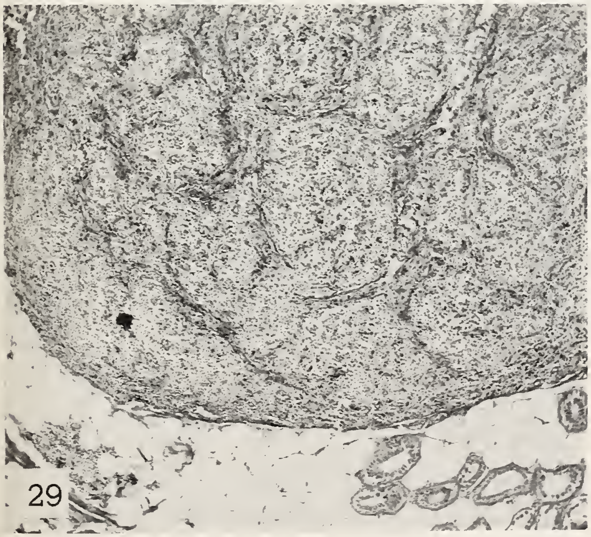
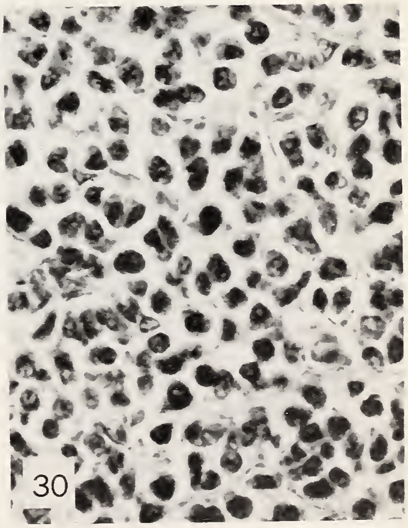


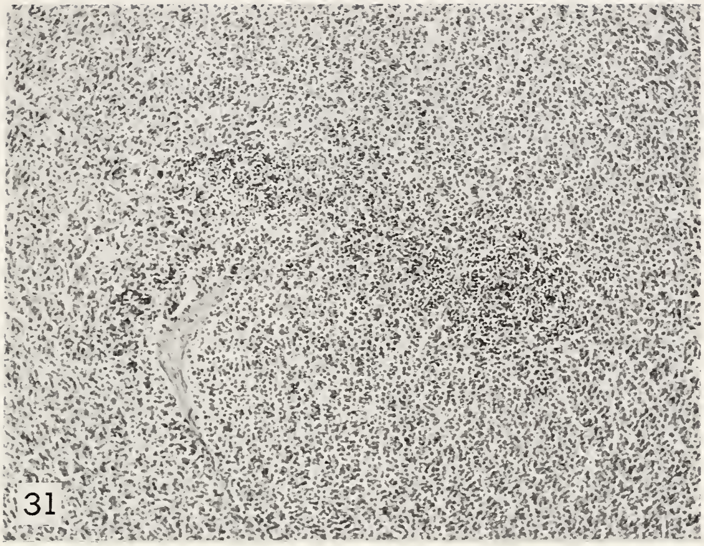
29



30

Figure 31.—Granulocytic sarcoma, lymph node, pig. *Note* diffuse infiltration of neoplastic granulocytes, leaving a small lymphoid focus and a fragment of trabeculum. Hematoxylin and eosin. Armed Forces Institute of Pathology (AFIP) negative 68-6492-31. × 80

Figure 32.—Higher magnification of figure 31, showing details of neoplastic granulocytes and delicate supporting stroma. Giemsa-stained slides showing eosinophilic cytoplasmic granules. AFIP negative 68-6492-32. × 600



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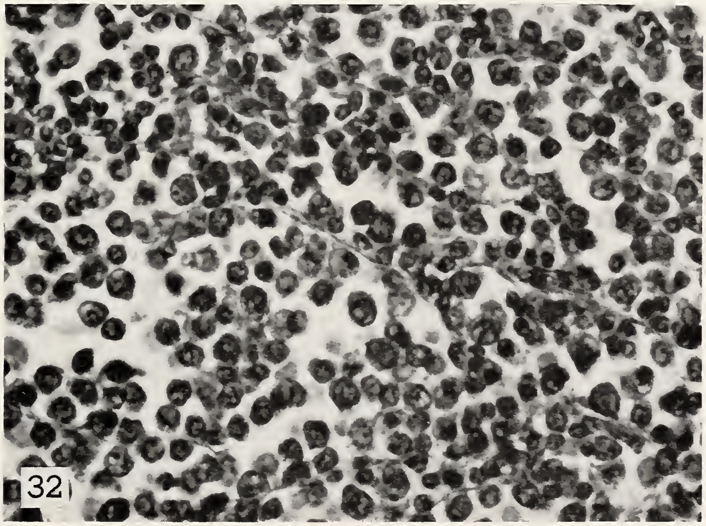

32

Spontaneous Hematopoietic Neoplasms of Free-Living and Captive Wild Mammals [1]

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SUMMARY

Knowledge of spontaneous neoplasms of the hematopoietic system in free-living and captive wild mammals has been reviewed and summarized. Neoplasms of this system have been recorded in one or more species of 8 of the 19 established orders of mammals. Material filed in the Registries of Veterinary Pathology and Comparative Pathology of the Armed Forces Institute of Pathology includes examples from 7 of the 8 orders. The increasing awareness of the importance of comparative aspects of human and animal medicine requires acknowledgment that the value of observations of diseases of any species is proportional to the completeness and availability of the material. Consequently, it is hoped that material from additional cases will be contributed to established registries of pathology so that information can be accumulated concerning diseases of animals of the rarer species.—Nat Cancer Inst Monogr 32: 153–156, 1969.

INFORMATION pertaining to neoplasms of free-living and captive wild mammals is scattered in many publications, including annual reports of zoological societies and a compendium of individual case reports. A search for records of spontaneous hematopoietic neoplasms in these animals reveals only a few reports in a small number of species of mammals.

This paper reviews the orders of mammals in which neoplasms of this system have been recorded in accessible publications, and furnishes a workable list of references, although it must be emphasized that many additional cases are recorded in obscure publications and in the files of zoological gardens.

To our knowledge, orders of mammals in which hematopoietic neoplasms have yet to be reported are Monotremata, Insectivora, Dermoptera, Chiroptera, Edentata, Pholidota, Cetacea, Tubulidentata, Hyracoidea,

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Sirenia, and the exotic members of the order Perissodactyla.The taxonomic classification is that of Walker (*31*).

Table 1 summarizes reported cases and cases on file at the Armed Forces Institute of Pathology. Discussion of each case will be omitted, since most reports consist of little more than a morphologic diagnosis and there is a striking absence of clinical and histopathologic descriptions. A few authors have summarized tumors of the hematopoietic system for a particular order or species. Lingeman *et al.* (*15*) have compiled a review of 22 examples in nonhuman primates. Van Kampen (*30*) has summarized reports in the rabbit. Halloran (*9*), Lombard and Witte (*16*), Ratcliffe (*23*), and Squire (*26*) have provided additional examples in animals of various species.

TABLE 1.—Summary of reported cases and cases on file at the Armed Forces Institute of Pathology

Order	Genus/species	Common name	Authority
Monotremata			No reports
Marsupialia	*Didelphis marsupialis virginianus*	Opossum	Lombard and Witte, 1959 (*16*) AFIP* Acc. No. 202373
	Phascolarctos cinereus	Koala	Backhouse and Bolliger, 1961 (*1*) Hill *et al.*, 1955 (*13*) Heuschele and Hayes, 1961 (*11*) AFIP Acc. No. 978624
	Sarcophilus harrisii	Tasmanian devil	AFIP Acc. No. 1171920
	Unknown	Dasyure	Sutton, 1885 (*27*)
Insectivora			No reports
Dermoptera			No reports
Chiroptera			No reports
Primates	22 reports, numerous species		DePaoli and Garner, 1968 (*4*) Lingeman *et al.*, 1969 (*15*)
Edentata			No reports
Pholidota			No reports
Lagomorpha	*Sylvilagus* sp.	Rabbit	Lopushinsky and Fay, 1967 (*17*) Van Kampen, 1968 (*30*) AFIP Acc. No. 972961
	Lepus sp.	Hare	Burgisser, 1957 (*2*) Guillon *et al.*, 1963 (*8*) Piening and Wermerssen, 1965 (*22*)
Rodentia	*Cuniculus paca*	Paca	Lombard and Witte, 1959 (*16*)
	Unknown	Cavy	AFIP Acc. No. 202374
	Chinchilla laniger	Chinchillas	Kast, 1962 (*14*) Newberne and Seibold, 1953 (*21*)
	Dipodomys ordii richardsoni	Kangaroo rat	Lombard and Witte, 1959 (*16*)
Cetacea			No reports
Carnivora	Unknown	Fox	Szuperski, 1954 (*29*) AFIP Acc. No. 124529, 190266

*Armed Forces Institute of Pathology.

Table 1.—Summary of reported cases and cases on file at the Armed Forces Institute of Pathology—Continued

Order	Genus/species	Common name	Authority
Carnivora—Con.	*Gulo gulo*	Wolverine	McFarland, 1961 (*19*)
	Tayra barbara	Tayra	Hill, 1951 (*12*)
	Paguma larvata	Civet	Lombard and Witte, 1959 (*16*)
	Mustela sp.	Mink	Møller and Heje, 1961 (*20*)
	Spilogale sp.	Skunk	Lombard and Witte, 1959 (*16*)
	Ursus sp.	Bear	AFIP Acc. No. 794777
	Mustela sp.	Ferret	Symmers and Thomson, 1953 (*28*)
Pinnipedia	*Neophoca* sp.	Sea lion	Engelbreth-Holm, 1942 (*5*)
	Phoca vitulina	Seal	AFIP Acc. No. 1256783
Tubulidentata			No reports
Proboscidea	Unknown	Elephant	Engelbreth-Holm, 1942 (*5*)
Hyracoidea			No reports
Sirenia			No reports
Perissodactyla			No reports
Artiodactyla	Unknown	Wild pig	Marienburg, 1966 (*18*)
	Numerous species	Deer	Debbie and Friend, 1967 (*3*) Engelbreth-Holm, 1942 (*5*) Hansen and Borg, 1966 (*10*) Lombard and Witte, 1959 (*16*) Woodford, 1966 (*32*) AFIP Acc. No. 1227864
	Alces alces	Elk	Engelbreth-Holm, 1942 (*5*) Hansen and Borg, 1966 (*10*)
	Oryx leucoryx	Oryx	AFIP Acc. No. 202380
	Numerous species	Buffalo	Engelbreth-Holm, 1942 (*5*) Ressang, 1957 (*24*) Singh, 1968 (*25*)
	Ovis aries	Sheep	Lombard and Witte, 1959 (*16*)
	Capra hircus	Goat	Fox, 1912 (*6*) Grunert, 1960 (*7*)

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Spontaneous Hematopoietic Neoplasms of Nonhuman Primates. Review, Case Report, and Comparative Studies[1,2]

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SUMMARY

Spontaneous leukemias and lymphomas have been recorded in 21 nonhuman primates. These include individuals of 5 of the 11 "nonhuman" families of this order and approximately 10 of the 60 genera. In two reports genera were not recorded. A majority were those species of Old World monkeys that are most numerous in captivity; hematopoietic neoplasms have been recorded in 2 rhesus, 2 cynomolgus, and 4 monkeys of the genus *Cercopithecus,* and a mangabey (genus *Cercocebus*). (Genus and species were rarely recorded; most were designated by common names.) The three examples in New World monkeys were a woolly monkey, a tamarin, and a marmoset. The only reports of hematopoietic neoplasms in higher apes were in 2 gibbons. An unconfirmed leukemia in a galago was the only example in any of the 6 families of prosimians. Most accounts were quite brief; for only 7 animals were descriptions of clinical and necropsy examinations given in enough detail to provide unequivocal evidence that a leukemia or lymphoma did in fact exist. Examination of material from 3 gibbons with spontaneous hematopoietic neoplasms from the Registries of Veterinary and Comparative Pathology of the Armed Forces Institute of Pathology, 2 of which have been described previously, provided an opportunity to compare characteristics of neoplasms of this group in one family of primates with those of man and other animals. A nodular, poorly differentiated lymphocytic lymphoma of a gibbon provided the third recorded example of a spontaneous hematopoietic neoplasm in a gibbon and was the 22d case report in a nonhuman primate.—Nat Cancer Inst Monogr 32: 157–170, 1969.

THE LACK of information concerning prevalence and morphologic characteristics of spontaneous hematopoietic neoplasms of nonhuman primates is in striking contrast to the abundant knowledge of neoplasms of this

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[3] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

[4] On leave as Pathologist for the Comparative Pathology Registry, Armed Forces Institute of Pathology.

[5] We appreciate contributions of histologic material from Dr. B. J. Cohen, Dr. V. B. Robinson, and Dr. R. DiGiacomo. We acknowledge assistance of Dr. Francis M. King and Dr. Roger O'Gara.

group in man and representatives of many other orders of mammals and birds. Table 1 lists features of the 21 recorded cases known to us compiled from the bibliographies of Ruch (*1*) and Luther (*2*) and from other sources, including a MEDLARS search.[6]

Most descriptions were very brief and did not include complete details of clinical and necropsy examinations. Many were included in reports of zoological societies. Probably there are additional ones in publications not readily available to us. We have attempted to contact authors of the most recent papers to request additional information and opportunity to examine histologic material. To date this search has yielded material from only 2 cases.

REVIEW OF LITERATURE

In 1923, Massaglia (*3*) described clinical features of a disease believed to be chronic lymphatic leukemia in a cynomolgus monkey of unknown age observed during experiments involving inoculations of materials from human patients with hematologic diseases, including leukemia. The diagnosis was based on observations of generalized lymphadenopathy, splenomegaly, anemia (Hb 55%), and leukocytosis (36,000/mm^3), of which 82% of cells were lymphocytes and other mononuclear cells. Details of necropsy, if performed, were not reported. The evidence from this brief description is inconclusive.

In 1929, Corson-White (*4, 5*) described in 2 reports a lymphoid neoplasm in a female green monkey (*Cercopithecus callitrichus*), age not stated. At necropsy, lungs, liver, spleen, lymph nodes, tonsillar regions, mucosa of stomach, and jawbone were infiltrated with peroxidase-negative mononuclear cells in a distribution characteristic of leukemia or lymphoma. Peripheral blood, obtained post mortem, contained 79% lymphocytes. The complete description provides convincing evidence of a lymphoma or leukemia.

In 1934, Noback (*6*) reported a neoplasm characterized as a "malignant round cell sarcoma" of axillary, inguinal, and abdominal "regions" in a male chacma baboon. Although a hematopoietic origin may be suspected, the description was too brief to confirm this impression.

In 1937, Oshima (*7*), in a series of necropsies of zoo animals in Japan, described a leukemic disease of a primate (species not designated) involving lung, liver, spleen, and lymph nodes. Infiltrating cells were believed to be of granulocytic origin. Although the term "myeloma" was used, plasma cells were not described. Use of the term "myeloma" to designate "solid" masses of leukemic cells of granulocytic origin in chickens can be traced to Furth (*8*).

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[6] MEDLARS (Medical Literature Analysis and Retrieval System), National Library of Medicine, Bethesda, Md. 20014.

TABLE 1.—Recorded spontaneous hematopoietic neoplasms of 22 nonhuman primates

Author	Number of animals	Common names	Sex	Age	Authors' diagnoses; comments
Massaglia, 1923 (*3*)*	1	Cynomolgus monkey	—	—	Lymphatic leukemia; necropsy not described.
Corson-White, 1929 (*4*, *5*)*,†	1	Green monkey	F	—	Lymphatic leukemia; necropsy described.
Noback, 1934 (*6*)	1	Chacma baboon	M	—	Round cell sarcoma; few details given.
Oshima, 1937 (*7*)	1	Unspecified primate	M	—	Myeloid leukemia; necropsy performed.
Krause, 1939 (*9*)†	1	Unspecified primate	—	—	Lymphosarcoma; brief mention only. Attributed to Bland-Sutton.
Hamerton, 1942 (*10*)*	1	Stair's monkey	—	—	Lymphatic leukemia; only brief description of necropsy.
Kelly, 1948 (*11*, *12*)*,†	1	Baboon	F	Adult	Hodgkin's sarcoma; diagnosis based on biopsy.
Hill, 1950–52 (*13*), and Fiennes (*15*) cited by Lapin (*14*)	4	Woolly monkey, galago, marmoset, tamarin	—	—	Leukemias; only brief descriptions.
Gillman and Gilbert, 1954 (*16*)†	1	Baboon	F	Adult	Reticulosarcoma; described only briefly.
Kent and Pickering, 1958 (*17*)*,†	1	Rhesus monkey	F	5 yr	Lymphoma; necropsy described.
Newberne and Robinson, 1960 (*18*)†	1	Gibbon	M	—	Lymphoma; necropsy described.
Gorlin, 1960,† cited by Cohen and Goldman (*19*)	1	Rhesus monkey	—	—	Lymphosarcoma; description incomplete.
Jungherr, 1963 (*20*)	1	Cynomolgus monkey	F	—	Lymphosarcoma; only kidneys examined.
Klüver, 1963, cited by Jungherr (*20*)	1	Sooty mangabey	—	—	Lymphosarcoma; mentioned only briefly.
Lapin and Yakovleva, 1963 (*14*, *21*)	3	African green monkeys	—	—	Reticulosis, hemoblastic leukemia.
DiGiacomo, 1967 (*22*)	1	Gibbon	F	4–5 yr	Lymphoma; necropsy described.
DePaoli and Garner, 1968 (*31*)	1	Gibbon	M	1½ yr	Acute leukemia.

*Cited by Ruch (*1*).
†Cited by Luther (*2*).

In 1939, Krause (*9*), in an extensive review of diseases of wild and captive animals, included mention of a lymphosarcoma of a primate, of uncertain species, attributed to Bland-Sutton.

In 1942, Hamerton (*10*) described naturally occurring diseases of 11 Old World monkeys in the London Zoological Gardens. One Stair's monkey (*Cercopithecus albogularis*) with a disorder of the central nervous system accompanied by dietary anemia was observed to have "lymphatic leukemia associated with hyperplasia of abdominal lymph glands." There were no other details.

A 1948 report from the San Diego Zoo included a statement by Kelly (*11*) that a tentative diagnosis of Hodgkin's sarcoma was made in a baboon after examination of biopsy tissue from an unspecified site. Pathologists who examined the histologic material from this procedure and subsequent necropsy are deceased (*12*). Histologic material is not available for review.

Reports from the Zoological Society of London listing leukemias as causes of death in 4 primates (*13*) have been cited by Lapin and Yakovleva (*14*). Fiennes (*15*) has been unable to confirm diagnoses of hematopoietic neoplasms in any primates of this collection.

In 1954, Gillman and Gilbert (*16*), of the University of Witwatersrand, South Africa, described a neoplasm of a female baboon that occurred 6 years after subtotal thyroidectomy. A reticulosarcoma was said to have involved lymph nodes and abdominal viscera. Our attempts to obtain additional information to supplement this brief description have been unsuccessful.

In 1958, Kent and Pickering (*17*) reported a spontaneous lymphoma of a female rhesus monkey approximately 5 years old. The diagnosis was supported by a complete description of clinical, hematologic, and necropsy observations. The clinical course was characterized by lymphadenopathy, leukopenia (2,300/mm^3) with lymphocytosis (88%), and anemia (Hb 4.3 g/100 cc). A lymph node biopsy and subsequent necropsy revealed extensive neoplastic infiltration of liver, spleen, kidney, and lymph nodes.

In 1960, Newberne and Robinson (*18*) observed a spontaneous lymphoma of a male gibbon of unknown age, based on examination of postmortem histologic material obtained from a veterinarian attending a zoo in Florida. Because a hematopoietic neoplasm was not suspected before death, a complete hematologic examination was not performed. Liver, spleen, kidney, lymph nodes, and myocardium were infiltrated by mononuclear cells believed to be of lymphocytic origin. Distribution of infiltrates was consistent with leukemia.

Also, in 1960, a personal communication from Gorlin was cited by Cohen and Goldman (*19*) as a source for information that a rhesus monkey had developed a lymphosarcoma of the lower lip. There were no other details.

In 1963, Jungherr (*20*) observed bilateral mononuclear cell infiltrates in kidneys from a young female cynomolgus monkey that had been killed as a donor for cell culture. Because a lymphoproliferative disease had not been suspected, other organs were not examined. Jungherr also cited a personal communication from Klüver, dated 1962, who observed a disease believed to be generalized lymphoma in a male sooty mangabey. There were no other details.

Also, in 1963, Lapin and Yakovleva (*14*), of the Sukhumi Primate Colony, Soviet Union, described generalized hematopoietic neoplasms in 3 monkeys of a colony of 47. Translations give conflicting designation of species. All were African green monkeys (*Cercopithecus aethiops*) (*21*). All had clinical courses characterized by acute onset and severe hypochromic anemia. In one, a diagnosis of reticulosis was based on necropsy observations, which included hepatomegaly, splenomegaly, renomegaly, and lymphadenopathy, associated with microscopic infiltrations of reticular cells in bone marrow, spleen, liver, kidneys, and lymph nodes. In the second monkey, acute hemocytoblastic leukemia was diagnosed, characterized by extensive infiltrations of lymphoid tissues by hemocytoblasts. In the third monkey (possibly described in a separate study that we were unable to locate) lymphogranuloma of liver and spleen was diagnosed. This unusual concentration of spontaneous hematopoietic neoplasms in a colony of primates represents the largest series reported to date. All occurred within a 6-year period.

In 1967, DiGiacomo (*22*) reported a malignant lymphoma of a female gibbon 4–5 years of age. A necropsy revealed dense neoplastic infiltration of lymph nodes, liver, spleen, and bone marrow. The impression that the neoplasm was comparable to the lymphoma of Burkitt and O'Conor (*23*) was based on presence of phagocytes with the appearance of "starry skies" amid monotonous sheets of cells believed to be of lymphocytic origin.

The small number of hematopoietic neoplasms of monkeys and apes that has been observed, compared to incidence of neoplasms of other cell types, is impressive. Lombard and Witte (*24*) found no hematopoietic neoplasms among 13 malignancies of other cell types in 1,328 primate necropsies performed during a 54-year period (1901–55) in the Philadelphia Zoological Gardens. Maruffo (*25*) reported 2 malignant neoplasms, neither of hematopoietic origin, in a colony of 292 howler monkeys. Lapin and Yakovleva (*14*) observed a malignant neoplasm in each of 2 baboons, 19 and 20 years of age, in a colony of 156, of which 10 survived more than 10 years. Neither tumor was of hematopoietic origin. No spontaneous malignant neoplasms were observed in 214 macaques, of which 15 survived for more than 15 years. The 3 leukemic neoplasms in primates of the Sukhumi colony occurred in monkeys of the genus *Cercopithecus*.

Reports from laboratories in which large numbers of young monkeys have been used for a variety of short-term experiments indicate that malignant neoplasms were rare in these animals. Tuberculosis and other infectious diseases accounted for a high mortality in animals that died of causes other than those related to experimental procedures. Kennard (*26*) did not observe neoplasms in 246 necropsies of monkeys. Habermann and Williams (*27*) found none in 708 necropsies of monkeys. Fairbrother and Hurst (*28*) did not encounter neoplasms in 605 necropsies of prepubertal monkeys.

More than 800,000 young monkeys, mostly rhesus, were imported into the United States from 1957 through 1960 for production and testing of polio vaccine (*29*). Mass-production requirements precluded performance of extensive clinical examinations and necropsies of animals that were retained for only a few weeks. Many were used only as donors for kidneys for cell cultures; the remaining organs were discarded. Newberne and Robinson (*18*) found only a single malignant neoplasm, a carcinoma of uncertain origin involving ovaries and uterus in a cynomolgus monkey. Jungherr (*20*) noted only a single example of a possible lymphoma of kidneys.

CASE REPORT AND COMPARATIVE STUDIES

The Registries of Veterinary Pathology and Comparative Pathology of the Armed Forces Institute of Pathology (AFIP) contain histologic material from only 6 examples of leukemias and lymphomas in a total of 14 malignant neoplasms from various species of nonhuman primates. Two are irradiation-induced granulocytic leukemias in rhesus monkeys, one of which has been reported by the contributor (*30*).[7] Two spontaneous hematopoietic neoplasms of gibbons specifically requested for this study have been reported by contributors (*18*, *22*). A necropsy of a gibbon that died of acute leukemia in the National Zoological Park in Washington, D.C., will be described by the prosector in a separate report (*31*). An additional example of a spontaneous lymphoma of a gibbon has not been reported and will be described in detail.

A 3½-year-old male gibbon developed nonspecific symptoms of illness approximately 1 month before death in 1955. At necropsy of the animal, the prosector noted evidence of weight loss, pallor, and generalized lymphadenopathy. The enlarged liver and spleen contained grossly visible nodules. Sections of liver, spleen, lymph nodes, lung, and kidneys were contributed to the Veterinary Pathology Registry of the AFIP.[8]

A nodular lymphoma involved lymph nodes, liver, and spleen. Lung and kidney were not infiltrated. In a section of lymph node, architecture was effaced by a nodular proliferation of predominantly small cells with dense nuclear chromatin and indistinct and scant cytoplasm (figs. 1 and 2). Nuclei of some cells were larger and more vesicular. Nucleoli were inconspicuous. Occasional cells contained intracytoplasmic erythrocytes, nuclear remnants, and particles positive for periodic acid-Schiff (PAS). Similar nodular foci were observed in the spleen (figs. 3 and 4). Erythrophagocytosis was more prominent in the spleen than in lymph nodes, particularly in sinusoids of areas not infiltrated by the neoplasm. The liver (figs. 5 and 6) was also infiltrated by neoplastic nodules similar to those observed in nodes and spleen, except that erythrophagocytosis was not so prominent. This neoplasm is considered to be a nodular, malignant lymphoma, poorly differentiated lymphocytic type, of lymph nodes, liver, and spleen, according to the classification of Rappaport (*32*).

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[7] Material from the second case was contributed by H. W. Casey, USAF, VC, Biosciences Division, USAF School of Aerospace Medicine, Brooks AFB, Texas.

[8] Contributed by Dr. Bennett J. Cohen, University of California Medical Center, Los Angeles, Calif.

Through the courtesy of authors of publications on spontaneous hematopoietic neoplasms of gibbons (*18*, *22*), we have had an opportunity to examine histologic material from 3 cases and to compare the cytomorphologic features.

The neoplasm of the gibbon reported by Newberne and Robinson in 1960 (*18*) has features consistent with a granulocytic leukemia, though neither peripheral blood nor bone marrow was available for examination. (The histologic material had been submitted to the contributing pathologists by a veterinarian attending a zoo; a hematopoietic neoplasm had not been suspected before death.) The infiltrates of mononuclear cells resembling myeloblasts or myelocytes were diffuse in sinusoids of liver and spleen. The follicular arterioles of the spleen were intact. In lymph nodes the infiltrate was diffuse in sinusoids; a few lymphoid follicles were intact. Diffuse infiltrates were also noted in the renal interstitium. Large numbers of cells similar to those of infiltrates were circulating in peripheral blood, as seen in blood vessels of lung and mesentery. These infiltrating cells were relatively uniform in size, with large vesicular nuclei, and some had prominent nucleoli. Cytoplasm was scant but well defined, and the cytoplasmic boundaries were distinct. Progressive maturation toward mature neutrophils was not observed. Moderate numbers of normoblasts were observed in infiltrates and peripheral blood. Although sections of bone marrow were not available for examination, all evidence points to a myeloproliferative disease, most likely a subacute granulocytic leukemia, though myeloid metaplasia or other forms of extramedullary hematopoiesis could not be excluded. The density and monotony of the infiltrates suggest a leukemia.

The neoplasm described by DiGiacomo in 1967 (*22*) involved liver, spleen, bone marrow, and lymph nodes. The contributor had noted effacement of architecture of lymph nodes by a dense infiltrate of small mononuclear cells with intervening phagocytic histiocytes, giving the appearance of a "starry sky." Our examination of material from the neoplasm revealed that the predominant cells were 8–10 μ in diameter, with eccentrically placed nuclei, densely clumped chromatin, and ovoid, strongly PAS-positive cytoplasm. We believe that the cells of this neoplasm are related to plasma cells and suspect that the gibbon had a hyperglobulinemia. The phagocytic histiocytes are similar to those described in lymphomas of African children (*23*) and of American children (*33*, *34*), in other forms of lymphomas of man and animals (*35–37*), and in active lymphoid tissues generally (*35*).

DISCUSSION

The order Primates is composed of 11 or 12 families, of approximately 60 genera, and of nearly 200 species (table 2) (*38*, *39*). Characteristics of members of the order represent a wide spectrum, ranging from the rodent-like tree shrew to man. No single characteristic, but certain anatomic and physiologic features in various combinations, identify members of the order and separate primates from other orders of mammals. Primatologists do not agree on precise classification of individual members of the order, nor indeed which species should be included in the order. The recently published classification of the Napiers (*38*) will be followed in this review.

The 22 recorded examples of spontaneous hematopoietic neoplasms in subhuman primates include animals from 5 families and approximately 10 genera (table 1). Only one, by an unconfirmed report, occurred in a prosimian, a galago (*13*, *15*). Most were in various genera of popular laboratory monkeys. We are not aware of reports of neoplasms of this group in apes other than gibbons.

TABLE 2.—Classification of living members of the order Primates [from (*38*)]

	Family	Number of genera	Common names
Prosimii	Tupaiidae	5	Tree shrews
	Lemuridae	6	Lemurs
	Indriidae	3	Indrises, sifakas
	Daubentoniidae	1	Aye-ayes
	Lorisidae	5	Lorises, galagos, pottos
	Tarsiidae	1	Tarsiers
Anthropoidea	Callitrichidae	5	Marmosets, tamarins
	Cebidae	11	Woolly monkeys, capuchins, spider monkeys, howlers, sakis, and others
	Cercopithecidae	14	Macaques (rhesus, cynomolgus), green monkeys, mangabeys, baboons, and others
	Hylobatidae	2	Gibbons
	Pongidae	3	Orangutans, chimpanzees, gorillas
	Hominidae	1	Man

All spontaneous hematopoietic neoplasms of nonhuman primates in the AFIP Registries occurred in gibbons, an unexpected concentration in members of a single family. Gibbons are considerably less numerous in captivity than monkeys and apes of other families. Gibbons were formerly classified in the family Pongidae with other apes (gorillas, chimpanzees, orangutans) (*39*). More recently, however, they have been assigned to a separate family, the Hylobatidae, which includes 2 genera, *Hylobates* and *Symphalangus* (*38*). There are probably 7 species within the genus *Hylobates* and 1 or 2 in the genus *Symphalangus*, though precise classification is uncertain. Evidence, based on examinations of serum albumins, indicates that modern gibbons became separated in the evolutionary line of descent that led to the advent of other apes and of man 10 million years ago (*40*).

Gibbons are smaller and more agile than other apes. They mature sexually in 6–10 years and have survived as long as 30 years in captivity. The habitat of free-living gibbons is limited to forested islands and the mainland of Southeast Asia (*39*). Chromosome complements of the 2 genera are 44 (*Hylobates*) and 50 (*Symphalangus*). Those of all other apes are 48 (*38*). Those of man are 46.

The 3 gibbons for which ages were known were prepubertal (1½, 3½, and 4–5 years old). In man, hematopoietic neoplasms are the most prevalent neoplasms in prepubertal children, may be present at birth, and account for one-half of all childhood malignant neoplasms (*41-43*). Human leukemias and lymphomas may occur at any age, but the incidence of most types increases with age. There are definite age-associated differences in cell types of leukemias.

Hematopoietic neoplasms of domesticated mammals, mostly nonleukemic lymphomas, occur most frequently in mature animals, though they have been observed in bovine fetuses and calves, kittens, and young swine (*44*). Some authors have reported differences in anatomic sites of involvement and in cytomorphologic types of lymphomas as a function of age in cattle (*45, 46*). Age-dependent differences have also been observed in hematopoietic neoplasms of mice (*47*) and of chickens (*48*).

The cytomorphologic types of the spontaneous hematopoietic neoplasms of the 4 gibbons in the AFIP Registries include 2 leukemias (acute lymphocytic and subacute granulocytic), a poorly differentiated lymphocytic lymphoma, and a plasmacytic neoplasm. Reports in the literature also indicate that a wide spectrum of cell types of leukemias and lymphoma, similar to those in man, has been observed in various species of nonhuman primates.

More information is needed to characterize the spectrum of hematopoietic neoplasms that can occur in the many species of nonhuman primates. Precise cytomorphologic classifications cannot be made, particularly in myeloproliferative forms, without opportunities to examine peripheral blood and bone marrow. There is a need for more information concerning clinical courses of the diseases.

The cooperative efforts of scientists of many disciplines will be required to provide material from a sufficiently large primate population to define prevalence and morphologic characteristics of their malignant neoplasms. Mechanisms for collection and evaluation of this much-needed information exist in the AFIP Registry of Comparative Pathology and in the World Health Organization International Reference Center for Comparative Oncology.

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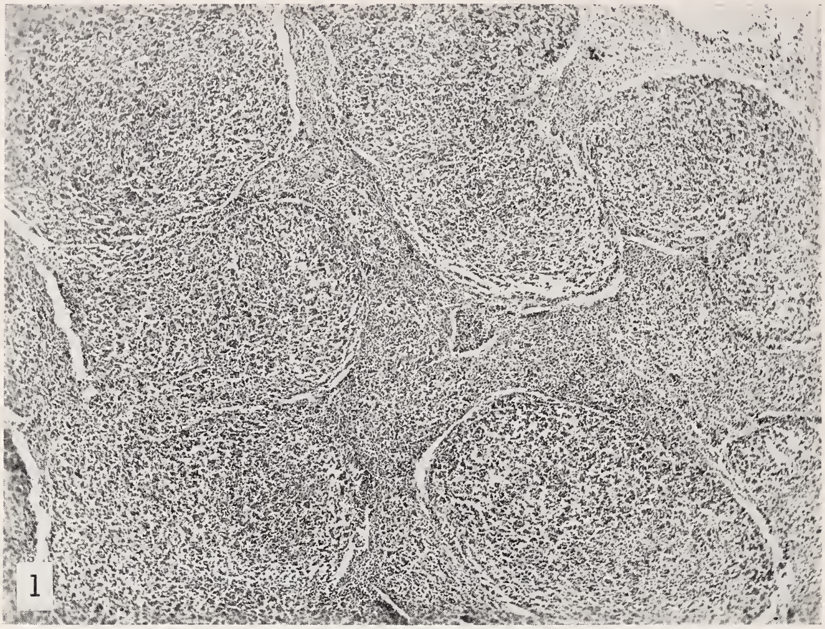
All photomicrographs stained with hematoxylin and eosin. AFIP = Armed Forces Institute of Pathology.



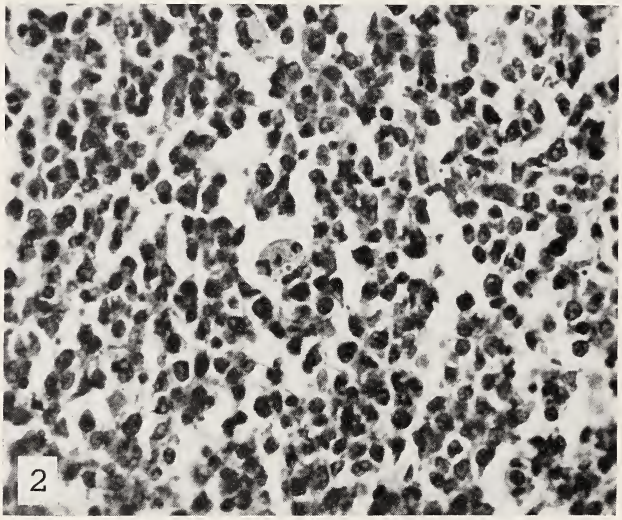
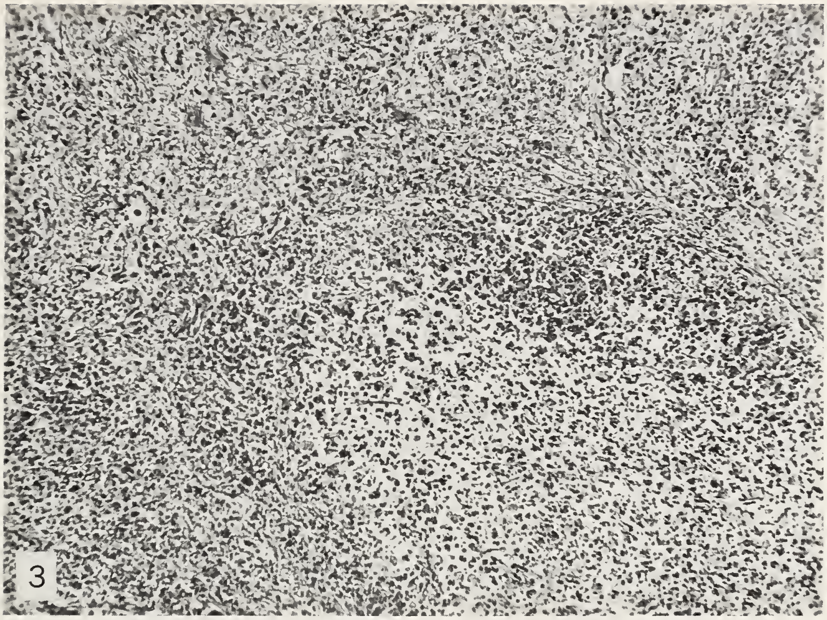


Figure 1.—Lymph node. AFIP negative #67-8467. × 40

Figure 2.—Lymph node. AFIP negative #67-8466. × 450



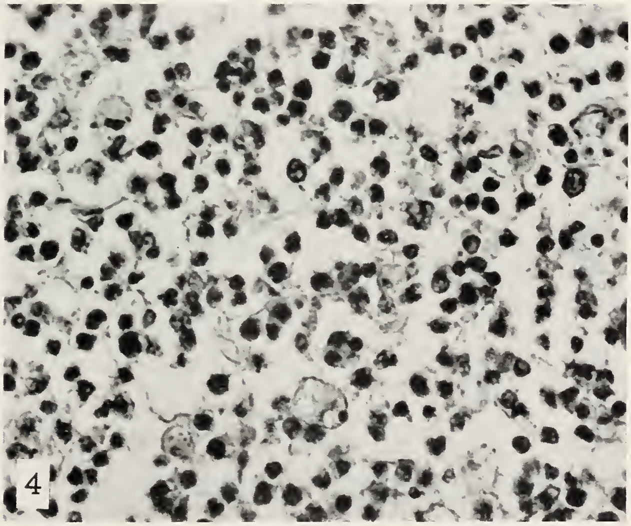
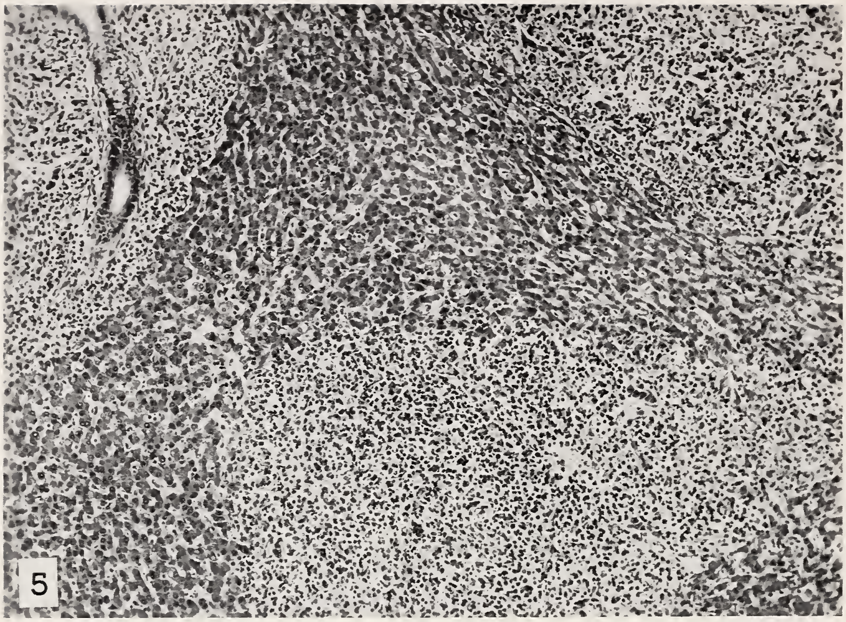


Figure 3.—Spleen. AFIP negative #68-4754. × 80

Figure 4.—Spleen. AFIP negative #67-8464. × 450



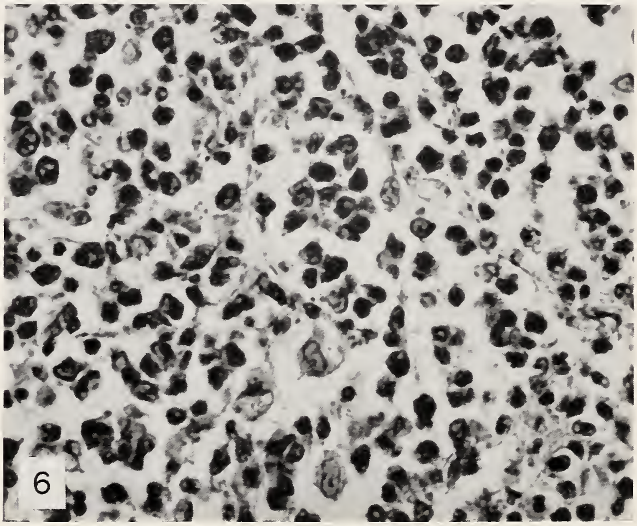


Figure 5.—Liver. AFIP negative #68-4755. × 80

Figure 6.—Liver. AFIP negative #67-8468. × 450

Comparative Aspects of Hematopoietic Neoplasms of Animals—Summary[1]

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OUR SESSION amply illustrates the growing appreciation by both medical and animal pathologists of the mutual advantages of meeting on the common ground of comparative pathology. The study of spontaneous, as well as of experimental, disease in animals already has contributed much—in fact, it is indispensable—to the progress of medical science, and in such fields as cancer research its greatest harvests still remain to be gathered. We are greatly indebted to the Armed Forces Institute of Pathology and the National Cancer Institute for the sponsorship of this Symposium and, in particular, to Col. Garner and Dr. Lingeman for the efficient detailed arrangements. Publication of the proceedings of Symposia can sometimes be of quite limited value, but in this instance the nature of our subject is such that the published, illustrated accounts will provide a handbook that is likely to be of lasting and increasing value to all working in our field of common interest. The various expert contributors are to be congratulated on their excellent efforts, and they can rest assured that their papers will be of great and continuing help to many beyond the confines of this present audience.

At this Symposium our central interest has been in morphology, though significant references to etiology, biology, and epidemiology have been made. A few years ago some pathologists believed that morbid anatomy in its morphological sense had perhaps come very near to the end of the road in human pathology. Although there was possibly at that time some basis for this feeling in respect to human hospital and autopsy pathology, it was certainly premature in respect to veterinary pathology, in which descriptive morphology still remained a subject largely unexplored in detail. However, recent advances in such fields as histochemistry and, in particular, the introduction of the electron microscope have shown that there are still important areas of morphology requiring detailed study in human pathology and, still more, in veterinary pathology.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] Professor Cotchin also served as Moderator for this session.

Morphology and function still remain the inseparable bases of pathology, and the pathologist's study of morphology enables him to define, in as precise and discriminating terms as possible, just what pathological condition is under consideration, whether from the point of view of epidemiology, etiology, experimental pathology, or other lines of investigation. In the particular conditions that are the subject of our Symposium, the present need is, in fact, for the strictest possible attention to morphology, so that we can keep our sense of direction in what otherwise appears to be a maze.

The object of comparative studies of pathology is to provide a deeper understanding of the disease processes in man and in animals by making relevant comparisons between the various species—and it was encouraging to hear from Dr. Squire about the postdoctoral training program in comparative pathology at The Johns Hopkins University. One difficulty that may hinder comparative studies arises from the fact that, as would be expected, far more is known about human pathology than about animal pathology. Many well-known difficulties beset a study of spontaneous neoplasms of domesticated animals, and *a fortiori* those of wild animals. Yet worthwhile work is being done in this field—due allowances being made for the facts that animals are not studied as closely as man is, many animals are killed young for food, and that sick wild animals cannot readily be necropsied, on the one hand, and, on the other hand, that veterinary pathologists are occupied with more obviously pressing problems.

There could be an argument for focusing the comparative pathologist's attention on conditions in animals that appear at first sight to be closely comparable to important conditions of man. This may limit attention to other conditions, which are apparently less immediately relevant, but which may warrant long-term study as significant pathological phenomena: *e.g.*, the leukosarcoma-like tumor occurring in parasitized Mayfly nymphs, mentioned by Dr. Dawe; the chromosome abnormality occurring in canine transmissible venereal tumors, referred to by Dr. Whang-Peng; and mast cell tumors of dogs, cats, pigs, cattle, and mice, referred to by Dr. Nielsen, Dr. Squire, Dr. Migaki, and Dr. Dunn. Further work needs to be done too on the chronic granulomatous condition of the spleen of the pig, also described by Dr. Migaki.

In assessing the scope of the subject of this Symposium—that is, hematopoietic neoplasms—it is very helpful to have a thorough appreciation of Dr. Rappaport's remarkable and lucid introductory statement in the Armed Forces Institute of Pathology Fascicle, "Tumors of the Hematopoietic System" (1966, pp 8–14). Dr. Rappaport reminds us that the hematopoietic tissues form part of a larger system which may be comprehensively termed "reticular tissue," the undifferentiated pluripotential cells of which are not confined to organs normally having well-defined lymphopoietic and myelopoietic tissues. These cells show hematic or stromal differentiation and give rise, on the one hand, to cells of the lymphocytic, erythrocytic, granulocytic, monocytic, and megakaryocytic series, and, on the other, to histiocytic and other tissue cells, including plasma cells and mast cells. If these facts are borne in mind, one can better understand the variable distribution and structure of neoplastic diseases of the reticular tissue. These facts also justify Dr. Dawe's claim that, although some metazoan invertebrates have no blood vascular system travelled by migratory cells, they should not therefore be excluded from discussion.

Neoplastic diseases of these tissues are classified primarily according to their cellular composition, with a subdivision, often very convenient clinically and grossly, into systematic proliferative diseases and initially localized tumors. In devising a nomenclature for the hematopoietic neoplasms of animals, we shall do well to have Dr. Rappaport's charts 1 (p 13) and 2 (p 98) constantly in hand, and indeed the whole Fascicle is an indispensable account of human neoplasms of this kind, against which animal tumors can be matched. If this Fascicle, or something like it, is eventually fully and widely accepted as the standard nomenclature for tumors of the hematopoietic system, comparative pathologists would do well to adapt their own nomenclature to it as nearly as possible, as this would greatly facilitate the exchange of information and ideas between workers in animal and human pathology.

However, it is important that this search for an approximation to human nomenclature should be conducted with the greatest possible critical assessment of the diseases occurring in animals, and the limitations of morphological similarity of lesions as confirming the identity of diseases in different species should not be forgotten. The question of whether Burkitt's lymphoma or Hodgkin's disease occurs in animals will not be answered on morphological considerations alone. Comparisons are drawn in various papers between human lesions and conditions in dogs (Dr. Squire), cats (Dr Nielsen), food animals (Dr. Migaki), rodents (Dr. Dunn and her colleagues), and other animals (Col. Garner and Dr. Lingeman); all will be of great help to medical and veterinary readers.

Morphologic descriptions can be provided at a number of levels—macroscopic, light microscopic, electron microscopic—and these descriptions can be supported by such techniques as those of histochemistry and immunochemistry. It has been repeatedly stressed in this Symposium, *e.g.*, by Dr. Nielsen and Dr. Squire, that optimum methods of fixation and preparation must be aimed at and, whenever possible, properly prepared and stained imprint smears should be studied.

Eventually a quantitative assessment of cytological features of these neoplasms will hopefully be possible. The pathologist often has to use such phrases as "the nucleus is somewhat paler" or "the cytoplasm has a rather more basophilic tinge" in his attempt to describe somewhat elusive cytological appearances. We must also not forget the importance of examining the blood picture and the bone marrow when assessing the nature of seemingly localized lesions. It is essential also that an element of time should be introduced where possible into our descriptions, so that a "four-dimensional morphology" evolves, which gives a temporal as well as spatial account of the lesions and provides a detailed analysis of such terms as "acute" or "chronic," "early," or "late," etc.

We also need to extend our knowledge of the structure of the hematopoietic and reticular tissues of animals. Veterinary hematology is a developing discipline, but more information is still required on the normal origin, function, and fate of the blood and reticular cells of domestic animals, and as for most wild animals, very little of course is known. While it seems reasonable to assume that morphologically similar cells will have biologically similar functions, this is not necessarily true in fact. In considering, for example, the relevance of avian leukosis to human medicine, the obvious differences in the red and white blood cells and the lymphatic tissues in these species must be remembered—the avian lymphocyte functions as the dominant cell in reactions to injury and infection (Dr. Helmboldt and Dr. Fredrickson)—and we should not overlook the normal lymphocyte predominance in the white blood cell picture of ruminants when we discuss bovine leukosis.

Similarly, Dr. Migaki has stressed what is only a too well-known difficulty of interpretation in studying lymph node morphology—how to distinguish between a neoplastic and a reactive proliferation (Dr. Dawe's remarks on tumor-like lesions associated with acid-fast organisms in amphibia are relevant here also). Again, further knowledge of the normal structure and responses of mammalian lymph nodes and of the lymphatic tissue in general in other species must influence interpretative appreciations of morphological changes.

Apart from inbred strains of mice, experience has already indicated that the lymphosarcoma is an important, if not the most important, tumor in a number of animal species. This tumor is strikingly frequent in the chicken and cat and it is of considerable significance in the dog and cattle. On cattle, of course, very extensive research programs are being carried out, stimulated by the apparent endemic occurrence of bovine lymphosarcoma in certain countries. The lymphosarcoma also forms a high proportion of neoplasms occurring in the horse, sheep, pig, and goat. It seems to be merely a matter of time before this tumor is found in any animal species in which there is lymphopoietic tissue comparable to that of mammals and birds, and in which a proper search has been made. Of interest have been descriptions of such instances as lymphosarcoma of *Mastomys* (Dr. Snell finding it in 10% of 200 old animals), of subhuman primates (Dr. Lingeman), of guinea pigs (Dr. Opler), of zoo and wild animals (Col. Garner), and even of certain lower animals (Dr. Dawe).

The apparent rarity of lymphosarcoma in rabbits is worth noting, as is the lack of reports, so far, of leukosis or hematopoietic neoplasms in reptiles or in cartilaginous (in contrast to bony) fish, although we should bear in mind Col. Garner's view that such tumors have not been reported in certain orders of animals because they have not been looked for. Dr. Squire has shown that a study of the correlation of cytological characters with therapeutic response is possible in the canine disease, using this convenient animal of suitable size and tractability. Dr. Nielsen has pointed out the advantages of a study of lymphosarcoma in cats and has stated: "The feline lymphosarcoma offers advantages over avian and murine laboratory models of leukemic neoplasms from the epidemiologic point of view. The house cat is exposed to the same radioactive and chemical pollutants in homes and streets as man, it eats similar diets including canned foods that have been sterilized and mixed with food additives and is inoculated with drugs and vaccines prepared in the same biologic systems as those for human use."

The various papers that have been given not only bring out the cytological details of the various diseases but also show the lesion variation that can occur both within and between species. Thus, lesions of the right heart, abomasum, and uterus seem to be characteristic of bovine lymphosarcoma (Dr. Migaki), and whereas this condition in dogs is often accompanied by a generalized enlargement of superficial lymph nodes (Dr. Squire), this is on the whole uncommon in the cat (Dr. Nielsen).

It has also been shown that occasional conditions occur in animals which do not seem to have a strict counterpart in man: *e.g.*, reticulum cell sarcoma type A (as described by Dr. Dunn in mice) and feline reticulosis (by Dr. Nielsen in cats), while mast cell proliferative diseases appear to be of great significance only in the cat and the dog. On the other hand, we still have to say that there has not yet been described any strict counterpart of Hodgkin's disease (*see* reports of Dr. Dunn on mice and of Dr. Migaki on granulomas of the spleen of the pig, and Dr. McIntire's account of the pleomorphic reticulum cell sarcoma in strain SJL/J mice, and the concomitant immunoglobulin abnormalities), or Burkitt's lymphoma (*note* Dr. Dawe's comment on the jaw lesions in pike) in animals.

The predominance of lymphopoietic neoplasms among hematopoietic tumors in so many species is a very striking observation and still requires explanation. In some species a viral etiology of this neoplasm is proved and in others is about to be proved, and it does not seem unduly rash to predict that viruses will eventually be found responsible for at least some forms of lymphosarcoma in most, if not all, species in which this disease occurs. We would then say that the prevalence of the tumor reflects the prevalence of the viruses, but we must be prepared to be surprised as investigations proceed (the complexity of the studies of the etiology of avian and murine leukosis is perhaps a paradigm of what may happen in respect to hematopoietic neoplasms in other species).

In the outcome, the continuing expenditure of time, effort, and money on the study of the comparative pathology of hematopoietic neoplasms will be justified if it helps provide solutions to problems relevant to human disease. Such questions as the following need answering, and have indeed been answered in part in this Session:

1) What neoplasms of the hematopoietic system occur in animals? As veterinary and other institutions gradually adopt data-recording and retrieval systems, information, particularly epidemiological, should become more rapidly available. Three particular institutions can be mentioned that will no doubt play an important role in collecting, analyzing, and disseminating material and information: *a*) the long-established Registry of Veterinary Pathology at the Armed Forces Institute of Pathology; *b*) the Registry of Tumors of Lower Animals at the Smithsonian Institution; *c*) the newly instituted World Health Organization Reference Centre for Comparative Oncology, also at the Armed Forces Institute of Pathology, and its collaborating Centres. The World Health Organization also has established a Collaborating Centre for Comparative Oncology for tumors of the hematopoietic system at the Glasgow University Veterinary School under Professor Jarrett, and it has sponsored international symposia on comparative leukemia research, in cooperation with the International Office of Epizootics. Veterinary liaison is also maintained with the World Health Organization International Reference Centre for the Nomenclature of (human) Leukaemias and Haematosarcomas, under Dr. Mathé.

2) How closely do animal neoplasms resemble morphologically and biologically human neoplasms of the hematopoietic system? The papers presented at this Symposium provide varyingly complete answers to this question.

3) Can we exploit observed similarities in studies of:

a) Etiology, using the term in a broader sense than the mere demonstration of a causal virus?

b) Biological behavior, including pathogenesis and clinical course?

c) Tumor therapy? Spontaneous tumors may well be superior to transplanted or induced tumors for such studies, but full information about the biological behavior of the types of tumor used will be needed to assist evaluation of therapeutic results.

d) Tumor prevention?

4) Do animal reservoirs of human hematopoietic neoplasms exist? Naturally some people have looked askance at animals as a possible source for origin of human leukemias, particularly those animals providing human food or biological products used in human medicine or animals living in close association with man at home or on the farm. The hypothesis that animals may be affected with an oncogenic agent capable of transfer to, and of effective action in, the human species must be treated at present with the greatest possible circumspection, but it seems to me that it cannot be summarily dismissed.

In the search for answers to such questions as these, the morphologist plays a central role, as he tries to say as clearly as possible just what pathological condition we are talking about. This Symposium will surely have clarified, extended, and sharpened the morphologist's vocabulary to advantage.

Epidemiologic Pathology of Leukemias and Lymphomas of Man [1]

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THE TERMS "leukemia" and "lymphoma" are often used indiscriminately and interchangeably to designate hematoneoplastic diseases of experimental animals. Observations of these diseases in man indicate that origin and pathologic progression of the two entities are different (*1*).

Leukemias originate in hematopoietic cell precursors of bone marrow or lymph nodes. Neoplastic leukocytes circulate in peripheral blood in early stages of disease. In contrast, *lymphomas* are initially localized tumors that originate in both hematopoietic and nonhematopoietic structures of the body. Dissemination by the blood or other route occurs as a secondary phenomenon. Lymphosarcoma cell leukemia designates circulation of neoplastic lymphocytes in peripheral blood of some patients with lymphomas.

Morphologic characteristics provide the major basis for classification of leukemias and lymphomas of man (table 1). *Leukemias* are classified by cytologic criteria based on examination of smears of peripheral blood and bone marrow stained by hematologic methods. *Lymphomas* are evaluated primarily by histologic criteria based on examination of fixed material. Diagnoses depend on *patterns* of cellular disorganization as well as morphologic features of proliferating cells. Some pathologists prepare imprints of lymph nodes or other structures, a cytologic technique that provides more information than histologic techniques alone. Clinical and epidemiologic considerations also contribute to pathologic evaluations of *leukemias* and *lymphomas*.

A wide range of cell types of leukemias and lymphomas is observed in man. The most frequent types fit into fairly well-defined clinicopathologic syndromes that can be analyzed by epidemiologic methods (*2*, *3*). This paper will be limited to descriptions of the more frequently occurring forms for which epidemiologic information has been accumulated.

Leukemias and lymphomas have been reported in many countries of the world but incidences of specific cell types are different in various popula-

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Table 1.—Morphologic classification of major hematoneoplastic diseases of man

Normal cell counterpart	Initially localized neoplasm	Primary leukemia
Undifferentiated hematopoietic cell	Lymphoma, undifferentiated	Undifferentiated leukemia (acute leukemia, stem cell leukemia)
Histiocyte	Lymphoma, histiocytic (reticulum cell sarcoma) Lymphoma, Hodgkin's type (Hodgkin's disease)	
Lymphocytic cells in stages of differentiation	Lymphoma, poorly differentiated lymphocytic (lymphoblastic lymphosarcoma) Lymphoma, well differentiated (lymphocytic lymphosarcoma)	Poorly differentiated lymphocytic leukemia (acute lymphocytic or lymphoblastic leukemia) Well-differentiated lymphocytic leukemia (chronic lymphocytic leukemia)
Hematopoietic cells:		
Granulocyte	Granulocytic sarcoma (chloroma)	Poorly differentiated granulocytic leukemia (acute granulocytic leukemia) Well-differentiated granulocytic leukemia (chronic granulocytic leukemia)
Megakaryocyte	Megakaryocytic myelosis	
Erythroblast	Erythremic myelosis	Erythroleukemia
Monocyte		Monocytic leukemias (acute and chronic)
Plasma cells	Plasmacytomas, plasma cell myelomas	Plasma cell leukemia
Mast cells	Mastocytosis	Mast cell leukemia

tions. Statistics for the United States and some European countries differ from those of some countries of Asia and Africa.

In the United States, deaths from all types of hematopoietic neoplasms are reported at an annual rate of 14.7/100,000 population (*4*, *5*). Approximately one-half are leukemias (7.0/100,000). The remainder are lymphomas (5.5/100,000), plasma cell myelomas (1.7/100,000), and miscellaneous entities (0.6/100,000). Only carcinomas of the large intestine (colon and rectum) and lung surpass neoplasms of the hematopoietic system in their lethal effects. Both leukemias and lymphomas can occur at any age but are most frequent in adults. Incidence of all types gradually rises with age through the seventh decade. Hematopoietic neoplasms, particularly acute leukemias, account for approximately one-half of all malignant neoplasms of prepubertal children in whom they cause death at an annual rate of 4/100,000.

Leukemias occur in definite age-associated distributions in the United States. *Acute leukemias* occur at all ages. Most leukemias of children are acute blastic leukemias. *Chronic granulocytic leukemia* is observed most frequently in young and middle-aged adults, but can occur during childhood. *Chronic lymphocytic leukemia* is a disease of individuals past 40 and is rarely observed in younger ages. Age-associated distributions have not been noted for other forms of leukemia.

With the exception of Hodgkin's disease and Burkitt's tumors, most lymphomas do not exhibit clear age-associated distributions. Lymphomas occur at all ages and there is a progressive increase in incidence with age. The special epidemiologic features of Hodgkin's disease and Burkitt's tumors will be described in a separate section.

For all age groups in the United States, lymphocytic types of lymphomas (lymphosarcomas) cause deaths with approximately twice the frequency of histiocytic types (reticulum cell sarcomas) (*4*, *5*). Individuals designated "nonwhite" have lower death rates from all types of hematopoietic neoplasms except multiple myelomas (*4*, *5*).

Statistics describing distributions of leukemias and lymphomas in Japan differ from those of the United States (*6–9*). Lymphocytic neoplasms, both leukemias and lymphomas, occur less frequently in Japan than in the United States. Reticulum cell lymphomas are more frequent than lymphocytic types (*6*). Hodgkin's disease occurs in different age distributions; the incidence peak in young adults in the United States is not observed in Japan (*7*).

Individuals of Japanese ancestry who have immigrated to Hawaii and their offspring have higher rates of leukemia than those living in Japan (*9*). This difference has not been noted for lymphomas. Such observations suggest that environmental influences are important in causation of leukemia.

Royston and Modan (*10*) compared incidences of leukemias and lymphomas in native-born and immigrant children in Israel. Those born outside of Israel had higher incidences of leukemia than the native-born, a difference that was not observed for lymphomas.

HEMATOPOIETIC NEOPLASMS

Burkitt's tumor (*11–14*) is the best known example of a lymphoma for which both age and geographic location are distinctive. Davies (*15*) compared and contrasted relative rates of childhood lymphomas in Nigeria and Uganda, countries which report high rates of Burkitt's tumors, with those of England and Norway. Lymphomas, predominantly Burkitt's tumors, accounted for 74 and 49% of all neoplasms in children in Nigeria and Uganda, while rates for both Norway and England were only 9%. In contrast, leukemias accounted for 28 and 35% of total childhood neoplasms in the two European countries, while in Nigeria and Africa they were only 4 and 6%.

ETIOLOGY

Although a variety of physical, chemical, and viral agents are known to be leukemogenic in animals, only irradiation in various forms and a few chemical agents, such as benzene, have been convincingly related to causation of leukemias and lymphomas of man.

Chemically induced leukemias are rarely recognized in man. Except for certain industrial exposures, man's "chemical environment" is complex, and attempts to relate onset of disease to definite exposures are usually unsuccessful.

Exposures to leukemogenic irradiation are readily documented. High incidences of leukemia are reported in radiologists compared to physicians of other specialties (*16, 17*), in infants exposed to maternal diagnostic irradiation while *in utero* (*18, 19*), infants irradiated for "enlarged thymuses" (*20, 21*), individuals of all ages following therapeutic irradiation for various reasons (*22, 23*), and those persons who were exposed to certain levels of radioactivity from the atom bombings (*24–27*). The leukemogenic effects of irradiation in these situations followed both single and multiple exposures to a wide range of sources and doses. In general, leukemogenic effects are most severe in younger individuals. Most leukemias of atom-bomb survivors have been granulocytic forms, both acute and chronic (*25*). Acute leukemias have also been reported following other radiation situations. *Lymphomas* were also observed in higher than normal incidences in Japanese atom-bomb survivors (*26*).

To date, no statistically acceptable epidemiologic evidence has been accumulated to indicate that human leukemias or most lymphomas cluster in households or communities except for a rare occurrence in more than one member of a family and the 25% concordance rates for monozygous twins for leukemia (*2*). Nor has development of human disease been convincingly linked to contacts with animals bearing similar neoplasms (*2*).

The importance of interactions of more than one etiologic event is emphasized in the report of Gibson and associates (*19*) who found that the highest risks of development of leukemia in children 1–4 years of age were those exposed to all of the four factors evaluated: mother's preconceptual irradiation, *in utero* irradiation of child, previous history of reproductive wastage, and early childhood virus diseases. Other combinations of irradiation and pathologic events increased the risk, but leukemia risk was not increased following a single event.

Such evidence supports a concept that leukemogenesis involves interactions of 2 or more events acting in "effective" sequences. The required sequences can be relatively specific or, more likely, can be effective in a limited number of alternate subsequences. The chance that a given individual will encounter the right combination in the right order is to some degree "random." The risk of leukemia can be increased or decreased by "natural" or "experimental" alterations in quantity or quality of one or more components of the sequence as genetic factors, age of individual, total of previous "immunologic experiences," dosage and site of contact with environmental leukemogens, and the order in which they are encountered. That all components of a leukemogenic sequence are met relatively infrequently explains why the concordance rate of leukemia in monozygous twins is only 25%, why a majority of individuals with Down's syndrome escape leukemia, and why most of us in fact do not develop leukemia.

Epidemiologico-pathological studies of spontaneous and experimental leukemic diseases of a wide range of species of animals and in various populations of man may identify additional components of the leukemogenic sequences, some of which hopefully can be controlled.

EPIDEMIOLOGIC PATHOLOGY OF LEUKEMIAS

The pathogenesis of leukemia regardless of cell type involves a progressive proliferation of immature leukocytes that circulate in blood and infiltrate bone marrow and other structures. The total leukocyte count is usually increased but may be normal or even decreased. Progressive anemia, hemorrhage, and susceptibility to infections mark the clinical courses of leukemias of all cell types, and signs of bone marrow failure are often the initial evidence of disease, particularly in acute forms.

Terminally, few structures escape the massive and destructive infiltrates of leukemic cells which are most conspicuous in bone marrow, liver, spleen, kidney, lymph nodes, gastrointestinal tract, heart, and lungs. Hemorrhages in vital structures such as the brain and/or overwhelming infections are usual causes of death. Massive infiltration of myocardium can cause heart failure. Infiltrates of leukemic cells in lungs may cause difficulty with respiration. Effects of infiltrates in other organs may have variable effects. All contribute to the general debility.

Opportunities to study the pathogenesis of leukemia during preleukemic phases are rare except for occasional patients with polycythemia vera who develop granulocytic leukemia. Block and associates (*28*) described

12 patients in whom acute leukemias followed protracted and nonspecific evidence of incipient bone marrow failure or hypersplenism, such as refractory anemia, hemorrhages associated with thrombocytopenia, and leukopenia. The true nature of the disease could not be determined during the preleukemic phases because the manifestations were nonspecific.

Despite similarities in pathologic processes which are common to all forms of leukemia, certain cell types of leukemia occur in syndromes with characteristic clinical, morphologic, and epidemiologic features that suggest differences in their etiopathogenic mechanisms. Descriptions of the many variants of leukemias that can occur are beyond the scope of this paper which will be limited to the most frequent forms.

Acute Leukemias

Neoplastic leukocytes of *acute leukemias* are usually undifferentiated or "blast" cells with few or no features suggesting differentiation toward established cell lines. In such instances, the disease is designated "acute blastic leukemia" or simply "acute leukemia." In other patients, leukemic leukocytes have features indicating their identity as myeloblasts, monoblasts, or lymphoblasts, and the appropriate name is incorporated into the diagnostic term.

Onset of acute leukemias, regardless of cell type, is usually rapid, although occasionally the prodromal phases are insidious. Initial signs are usually related to early bone marrow replacement, such as overwhelming infection incident to a decrease in leukocyte precursors or thrombocytopenia with hemorrhage. Anemia secondary to marrow erythrocytic cell replacement is often severe at the time of diagnosis. The profound debility of some patients with acute leukemia is frequently out of proportion to the severity of clinical signs.

Remissions following therapy by chemotherapeutic and supportive agents are usually dramatic but short-lived. However, in specialized leukemia treatment centers with multiple drug therapy, many remissions last beyond 1 or 2 years. Death frequently occurs within 1 year of onset, often within a few weeks or months, and is usually attributed to hemorrhage in a vital structure, such as the brain, or infection by viral, bacterial, fungal, or protozoal agents. The widespread infiltrates of leukemic leukocytes spare few structures in the terminal phases. Involvement of vital organs, such as the heart, liver, lungs, and kidneys contributes to the terminal debility.

Acute leukemias occur at all ages and can be present at birth. They are the most frequent neoplasms of prepubertal children in the United States (*4*, *5*) and Denmark (*29*). They account for 90% of all hematopoietic neoplasms of American children under 10 years of age. Peak age at death is 3 or 4 years. In contrast, leukemias are rare in children of certain areas of tropical Africa, such as Uganda and Nigeria, in which Burkitt's lymphoma is the most common childhood neoplasm (*15*, *30–32*).

Chronic Granulocytic Leukemia

The neoplastic leukocytes of *chronic granulocytic leukemia* are recognizable as myelocytes or related forms. Granules of leukemic cells are usually neutrophilic, but eosinophilic and basophilic myelocytes may be mixed with neutrophilic myelocytes or may even predominate. In such instances, the leukemias may be designated *eosinophilic* or *basophilic leukemia.* Increased numbers of megakaryocytes may be observed in bone marrow of some patients with chronic granulocytic leukemias.

Leukocyte counts in chronic granulocytic leukemia are among the highest of any disease and usually exceed 100,000/mm^3 in untreated patients. Liver and spleen are diffusely infiltrated; spleens can be massively enlarged and may weigh several thousand grams. Lymph nodes are inconspicuous and if involved are diffusely infiltrated; the architecture, including the follicles, is characteristically preserved.

The only consistent chromosomal anomaly that has been demonstrated in a hematopoietic neoplasm is the Philadelphia chromosome (Ph^1) that has been observed in blood cells of 90% of patients with chronic granulocytic leukemia (*33, 34*). This anomalous formation of a single chromosome is observed only in cells of blood and bone marrow, not in other cells, and is acquired rather than inherited (*35*). There is evidence that patients with chronic granulocytic leukemia who do not have the Ph^1 chromosome have a poorer prognosis than those in whom it is present (*34*). Young children did not have the Ph^1 chromosome in one series (*34*).

Survival of patients with chronic granulocytic leukemia is ordinarily less than 2 or 3 years following diagnosis. As in acute forms of leukemia, death occurs as a result of hemorrhage or infection incident to replacement of bone marrow elements. Widespread infiltrations of heart, liver, and kidneys contribute to the debility. A phenomenon which heralds the terminal phases is the so-called "blastic crisis"; the bone marrow becomes "packed" with immature cells that may be mistaken for "reticulum cells," and reports in the literature of lymphomas complicating chronic granulocytic leukemia may be examples of this phenomenon.

Chronic granulocytic leukemias have been reported from most areas of the world. They occur in individuals of all ages but are more frequent in adults, although significant numbers are observed in children and adolescents. Incidence increases gradually during young adulthood and rises steadily with age thereafter.

Granulocytic Sarcomas

Granulocytic sarcomas (chloromas) are invasive collections of immature granulocytes in bone or, less commonly, other sites, which are occasionally observed in patients with granulocytic leukemia, particularly the acute form (*1*). This manifestation of leukemia is rare in the United States and is observed more frequently in children than adults. Occasionally the sarcoma antedates the leukemia. Gross tumors are green because of verdoperoxidase or myeloperoxidase in granulocytes (*1*).

Davies and Owor (*36*) have reported that chloromas apparently occur more frequently in Ugandan than in European children. Chloromas were the most frequent initial presentation of myeloid leukemia and were observed in 8 of 13 children with myeloid leukemias observed in the Government Laboratory at Kampala, Uganda, between 1948 and 1964. All occurred in bones: the orbit in 6, occipital bone in 1, and tibia and elbow in 1. Ages of the 8 patients ranged from 3½ to 12 years. All patients had frankly leukemic blood.

Chronic Lymphocytic Leukemia

Neoplastic leukocytes of *chronic lymphocytic leukemia* are *well-differentiated lymphocytes.* A blood lymphocytosis may be the only evidence of disease and may antedate other signs by months or years. Patients may be asymptomatic and the leukemia may be discovered during a routine health examination. Survival is usually longer than for other forms of leukemia and may be 10 or even 15 years following diagnosis.

The process appears to begin in lymph nodes with complete replacement of nodal structures by mature lymphocytes. Such nodes cannot be distinguished from those of well-differentiated lymphocytic lymphomas by histologic examination alone. Bone marrow infiltration and replacement appear to occur as secondary events. Eventually infiltrates involve liver, spleen, and other structures. Those in the liver tend to be focal in portal areas, although some diffuse sinusoidal infiltration is observed at necropsy. Hemolytic anemia or other evidence of hypersplenism may occur during the course of disease (*37*). Some patients form abnormal immunoglobulins (*38*).

Chronic lymphocytic leukemia is a disease of adults over 40 years and is rarely observed in younger individuals. It is one of the most common forms of leukemia in the United States and some European countries but is rare in Japan (*9*).

Childhood Leukemia

Leukemias of children differ from those of adults in several respects. Most childhood leukemias are acute leukemias. Chronic granulocytic leukemia occasionally occurs. True chronic lymphocytic leukemias are not observed in children.

Whang-Peng and associates (*34*) reported that a higher incidence of Ph^1-negative chronic myelogenous leukemias occur in children and suggest that "juvenile" chronic myeloid leukemia may be a separate variant from the adult form. Of three children with chronic myelogenous leukemias studied at the National Institutes of Health, all were Ph^1-negative; the youngest Ph^1-positive patient was 9 years.

Leukemias of children with trisomic form of Down's syndrome tend to be *acute leukemias.*

Neonatal Leukemia

Leukemia may occur in the neonatal period and be present at birth. At least 50 cases have been reported (*39*). Most have been acute leukemias. Leukemic cell infiltration of the skin is a particularly prominent feature of neonatal leukemia and is distinctly uncommon at other ages. There are two reports of neonatal leukemias in offspring of leukemic mothers (*40–42*). Onset of acute lymphocytic leukemia in one mother (*40*) occurred during the 7th month of pregnancy, although the diagnosis was not established until 7 days post partum. The infant did not develop signs of leukemia until 9 months of age. Although a temporary drug-induced remission occurred in the infant, death occurred subsequent to the report (*41*). The bone marrows of both mother and infant were similar cytologically (*41*).

Bernard and associates (*42*) reported acute leukemia, probably lymphoblastic, in a 5-month-old infant born to a 19-year-old mother in whom a diagnosis of acute leukemia was made at the time of delivery of the infant. The mother succumbed to the disease 5 months after delivery; the infant was well and developed normally until 5 months of age, when signs of leukemia were first observed.

Diamandopoulos and Hertig (*43*) reported long-term follow-ups of offspring born of 33 women with leukemia or related diseases. None of 34 offspring of 48 births who survived the neonatal period developed leukemia. Some offspring were observed as long as 18 years. Twelve of 14 infants who died received necropsy examinations. None had leukemia.

Hoover and Schumacher (*44*) reviewed the literature on 59 cases of leukemia in pregnancy and one case in a patient of their own. They found only one report of leukemia in an infant born to a leukemic mother, the case of Cramblett and associates (*40*).

EPIDEMIOLOGIC PATHOLOGY OF LYMPHOMAS

Classification

Lymphomas are malignant neoplasms that originate in lymphoid or nonlymphoid structures from *reticular* or *lymphocytic* cells or their *undifferentiated progenitors* (table 1).

Undifferentiated lymphomas consist of cells with no morphologic features that suggest differentiation toward mature cell lines. *Burkitt's tumor* is considered to be one type of undifferentiated lymphoma.

Histiocytic lymphomas (*reticulum cell sarcomas*) consist of cells with one or more features of differentiated histiocytes, as reticulin formation or phagocytosis.

Hodgkin's disease is considered to be a neoplastic disease of histiocytes with a prominent inflammatory component.

Lymphocytic lymphomas (*lymphosarcomas*) consist of cells with morphologic features of lymphocytes in various stages of maturation; they are named according to the degree of differentiation of component cells.

Follicular lymphoma designates a histologic pattern in which neoplastic nodules or follicles mimic benign follicular lymphoid hyperplasia. The nodular pattern is associated with relatively benign behavior; conversion from a nodular lymphoma to a diffuse pattern is associated with increasingly malignant behavior, and progression of disease is similar to any diffuse lymphoma.

Pathogenesis

Lymphomas of all histologic types originate most frequently in a single lymph node or group of nodes, or less frequently, in an extranodal site. Progression of primary nodal disease may be unilateral or bilateral and may occur by extension to contiguous nodes or by metastasis to distant sites. For example, a lymphoma may begin in one or more superficial nodes of the lateral neck, progress to involve nodes of the thorax, and eventually cross the diaphragm to the retroperitoneal and abdominal nodes. Or the process may begin in nodes of the abdomen, thorax, or other site. Regardless of initial site, lymph nodes of most groups in the body and many viscera can be involved as the disease progresses. Despite complete architectural effacement and enlargement, involved lymph nodes tend to retain their "nodal" shape. They may be discrete, or groups of contiguous nodes may adhere to each other or to surrounding structures. They may be rubbery or firm and are particularly dense in fibrosing forms of Hodgkin's disease. Therapeutic procedures, particularly irradiation, can cause fibrosis and distortions of both neoplastic and non-neoplastic structures.

Primary nodal disease, if not halted, typically extends to non-nodal sites, and in terminal phases few structures escape involvement. Liver, spleen, kidneys, adrenals, lungs, and many other structures can be infiltrated by either grossly visible, discrete focal metastases or diffuse infiltrations. It is not unusual to find both patterns in a single organ, such as the liver.

Microscopic evaluation of a lymph node suspected of harboring a malignant lymphoma is one of the most difficult tasks a pathologist has to perform. Criteria for malignancy of nonhematopoietic cancers are difficult to apply to lymph nodes because cellular atypia and invasiveness are normal attributes of cells of the lymphoreticular system and benign hyperplasia can mimic neoplastic proliferation. It is difficult to map the pathogenesis of a lymph node undergoing neoplastic transformation and the process cannot be identified with certainty until disorganization or effacement has occurred. Descriptions of the many different appearances of lymph nodes involved by different cell types and histologic patterns of lymphomas are beyond the scope of this paper, and Rappaport's monograph (*1*) should be consulted for complete descriptions and illustrations.

Less frequently, lymphomas are extranodal in origin. Non-nodal structures that are most frequent sites of primary lymphomas are the stomach, intestines, tonsils, and other structures of the oronasopharynx, bone, or, less frequently, the lung, thyroid, gonads, kidneys, breast, skin, and salivary glands. Histologic patterns of extranodal lymphomas do not differ significantly from those originating in lymph nodes. Progression of visceral lymphomas is usually by way of regional lymph nodes, and examination of surgically removed organs reveals involvement of regional lymph nodes in many patients who do not have evidence of disease elsewhere.

The concept of initial localization of lymphomas is supported by experimental evidence. Avian leukosis originates in follicles of the bursa and can be prevented by bursectomy (*45*). Lymphomas of mice of certain strains can be prevented by thymectomy (*46*).

Initial localization of lymphomas of human patients is indicated by reports of "cures" following surgical removal, with or without irradiation, of primary tumors of the stomach, intestine, tonsil, kidneys, lymph nodes, bone, and occasionally other sites (*47–64*). Apparent cures have followed surgical excision of intestinal lymphomas of cats (*65*).

Wright (*66*) compared anatomic distributions of lymphomas of children in England with those in areas of Africa in which Burkitt's tumor is prevalent; most lymphomas of English children involved lymph nodes or other frankly lymphoid tissues, while lymphomas of African children were predominantly extranodal presentations. Migaki (*67*), Anderson *et al.* (*68*), Nielsen (*65*), and Squire (*69*) have emphasized that modes of presentation and anatomic distribution of lymphomas in animals of different species tend to manifest as definite nodal or non-nodal syndromes. The visceral lymphomas of the cat, which may be mediastinal or abdominal–visceral, and tend to spare peripheral nodes (*65, 68, 69*), are quite different from the common presentation of dogs in which symmetrical external lymph node involvement is prominent (*70*), although the canine disease may occur in a "visceral" form (*68*). In cattle the pattern is both nodal and visceral; involvement of the heart, abomasum of the stomach, and uterus appears to occur with unusual frequency in this species (*67*).

Survival

Survival of patients with lymphomas depends on many factors, including predominant site or sites of involvement in initial phases, extent of disease at diagnosis, cytologic type and histologic pattern, age, sex, and other characteristics of patients, therapy, and other variable factors.

Gall (*71*) and Gall and Mallory (*64*) analyzed survivals of patients with lymphomas according to site and histologic patterns. Twenty of forty-eight patients treated by surgical procedures, some of whom also received irradiation, survived without evidence of residual disease from 3–19 years (*71*). Three lymphomas were primary in the stomach, 7 in lymph nodes (cervical, axillary, inguinal), 5 in bone, 2 in the parotid gland, 2 in skin, and 1 in an eyelid. All major histologic types were represented among survivors. Peripheral node involvement was prominent in 90% of patients (*64*). Rosenberg *et al.* (*72*) reported 2 long-term survivors of 8 children, ages 2–13, whose lymphomas were treated by surgical procedures and irradiation. Two girls, ages 11 and 13, survived 15 and 13 years, respectively, without evident residual disease. Both lymphomas were reticulum cell sarcomas, one of neck nodes and the other of a fibula.

Both histologic pattern and extent of disease at diagnosis influence survival of patients with Hodgkin's disease (*73, 74*) and other lymphomas (*75*). Extent of disease is determined by several techniques, including lymphangiography. As anticipated, lymphomas confined to a definable and accessible "primary" site offer best chances for long survival of the patient. Survivals of Ugandan children with Burkitt's lymphoma can also be correlated with extent of disease at the time of diagnosis (*76*).

Studies based on material from 11 cancer registries in the United States have indicated an overall 5-year survival in from 18–42% of patients with lymphomas (*77*). Survivals tend to be longer for lymphosarcomas than reticulum cell sarcomas. Patients with the better differentiated lymphocytic lymphomas live longer than those with less differentiated forms. Women with Hodgkin's disease tend to live longer than men. There is no consistent correlation of longevity with age of patient.

Table 2 shows that survival correlates with both histologic type, site, and extent of disease (*78*).

TABLE 2.—Survival of 1,659 patients with lymphomas (*78*)

Histologic type	Number of patients	Percent of patients who survived		
		2½ years	5 years	10 years
Giant follicular lymphoma				
Localized	40	76	70	43
Disseminated	75	70	46	19
Lymphosarcoma				
Localized				
Non-nodal	62	43	33	27
One node group	72	72	65	44
Disseminated	207	38	25	12
Leukosarcoma	56	39	27	0
Reticulum cell sarcoma				
Localized				
Non-nodal	161	38	33	24
One node group	91	56	36	29
Disseminated	301	27	23	2
Hodgkin's disease				
Localized				
Neck nodes	125	87	70	57
Other	71	72	56	37
Disseminated	398	55	36	20

Survival correlated with extent of disease in 1,659 patients with lymphomas (*78*) (table 2). Ten-year survivals were highest in patients with Hodgkin's disease localized to lymph nodes of the neck (57%). Long-term survivals were also observed in patients with localized giant follicular lymphomas (43% survived 10 years) and lymphosarcoma localized to one group of nodes (44%). For both Hodgkin's disease and lymphosarcoma, localization in lymph nodes offered better survivals; for reticulum cell sarcomas this distinction could not be made. For all groups, evidence of dissemination at the time of diagnosis indicated a relatively poor prognosis; few patients with disseminated lymphosarcoma or reticulum cell sarcoma were alive after 10 years. The outlook was somewhat more hopeful in the other categories.

Lymphomas of Extranodal Origin

Experiences of two large American tumor registries (*78, 79*) indicat that lymphomas account for significant proportions of primary malignant neoplasms arising in such sites as the tonsil [11.8% of all malignant neoplasms of the site (*47*)], hypopharynx [3.5% (*80*)], small intestine [16–22% of all malignant neoplasms (*79, 81*)], stomach [1.1–2.5% of all cancers (*52, 79, 81*)], large intestine [0.3–0.6% of cancers (*79, 81*)], other structures of the oronasopharynx, gonads, bone, lungs, skin, kidneys, and other sites listed in table 3. Comparisons of the pathologic progressions of lymphomas located in various sites may provide insights into etiologic mechanisms of some diseases of this group.

Oronasopharynx

Jackson, Parker, and Brues (*47*) reported that 28 (11.8%) of 236 malignant neoplasms of tonsils were malignant lymphomas, predominantly lymphocytic and reticulum cell forms. Four patients had Hodgkin's disease. Five patients survived 7–18 years following surgical removal and/or irradiation. Fifty-six percent of patients developed generalized disease.

Oropharyngeal or nasal tumors were the presenting manifestations of 6.2% of lymphomas of adults and children recorded by Rosenberg *et al.* (*72*). Dockerty *et al.* (*80*) reported that lymphomas account for 3.5% of all tumors of the hypopharynx.

Primary lymphomas have reportedly originated in the mouth, tongue, nasopharynx, and salivary glands.

Gastrointestinal Tract

The stomach and intestines are frequently involved by lymphomas as part of a generalized process or, less commonly, are primary sites of origin. Primary gastric or intestinal lymphomas may remain localized for periods long enough to allow appearance of symptoms and opportunities for cure.

TABLE 3.—Primary lymphomas of selected extranodal sites expressed as percent of all malignant neoplasms of that site

Site	Connecticut Tumor Registry (*79*)	Memorial Hospital Registry (*78*)	Other sources
Oronasopharynx	1. 9		
Oral mesopharynx (includes tonsil)	12. 0		
Tonsil		10. 2	11.8 [Jackson *et al.* (*47*)]
Tongue		0. 8	
Nasopharynx and nose	5. 6	9. 0	
Hypopharynx			3.5 [Dockerty *et al.* (*80*)]
Respiratory tract (trachea, bronchi, lungs)	1. 0		
Lungs	0. 4	0. 5	
Gastrointestinal			
Stomach	1. 1	4. 0	2.5 [McNeer and Berg (*52*)]
Small intestine	22. 1	15. 6	20.0 [Berg (*81*)]
Colon and rectum	0. 6	0. 3	0.3 [Berg (*81*)]
Genital			
Testis	0. 8	2. 0	
Ovary	0. 1	0. 0	
Uterus	0. 1	0. 0	
Breast	0. 1	0. 5	
Glands			
Thyroid		2. 0	
Salivary	3. 4	8. 2	
Bone		11. 0	3.4 [Dahlin (*89*)]
Skin	0. 6		
Kidney	0. 2	0. 0	
Eye (includes orbit)	2. 0		

Berg (*81*) summarized features of 362 primary lymphomas of the gastrointestinal tract. He separated *primary lymphomas* which arose in the submucosa from those that were secondary to processes arising elsewhere in which invasion of the walls began at the serosal surfaces. Of primary gastrointestinal lymphomas of adults, 69% were located in the stomach, 24% in the small intestines, and 7% in the large intestines. In contrast, of primary gastrointestinal lymphomas of 23 children, 87% were located in the small intestines, and 13% in the large intestines; none were primary in the stomach. Of all intestinal lymphomas, 58% were located in the ileocecal region. Most lymphomas of the cecum also involved the appendix, but in no case did the appendix appear to be the primary site. Sixty percent of primary gastric lymphomas and 30% of those originating in the intestines were found in more than one site within the involved organ. Cure rates for primary gastric lymphomas following surgical removal, with or without irradiation, approached 32%, and prognosis was best for those that were clearly lymphocytic (*52*). The cure rate for intestinal lymphomas of both children and adults was nearly 20% (*81*).

Wychulis, Beahrs, and Woolner (*59*) reported that, of 50 patients treated by surgery and irradiation for primary lymphomas of the colon, 19 survived without recurrence for periods ranging from 3–55 years.

Regional lymph nodes were involved in 25 of 48 patients. Twenty lymphomas were classified as "mixed cell types," 16 were reticulum cell sarcomas, 12 were lymphocytic lymphomas, and 2 were Hodgkin's disease. Twenty-six patients had primary cecal lymphomas, which in 15 patients had extended into the terminal ileum. One lymphoma originated in the appendix. Ages of patients ranged from 4.5–82 years, and 11 patients were younger than 30 years. Chronic ulcerative colitis had been present in 3 patients for 5, 9, and 23 years. There are 7 reports in the literature of lymphomas occurring in intestines which were the site of chronic ulcerative colitis (*59*, *82*).

Primary intestinal tumors appear to occur more frequently in cats than other domestic animals (*65*). The abomasum of the stomach of cattle (the fourth or true digestive portion in ruminants) is frequently a prominent site of lymphomas, usually as part of a generalized process; it is not known whether this organ is ever a primary site (*67*, *83*).

Kidneys

Kidneys are frequently infiltrated in leukemias and disseminated lymphomas. *Primary* renal lymphomas are seldom recognized. Of 80 surgically removed primary sarcomatous renal tumors reviewed by Farrow *et al.* (*62*), all in adults, 10 were lymphomas and 1 was a plasma cell tumor. Of the lymphomas, 7 were called reticulum cell sarcomas, 1 a lymphosarcoma, and 1 Hodgkin's disease. The histologic type of the tenth case was not stated. In 8 patients, surgical exploration or X-ray examination of the contralateral kidney indicated that the process was unilateral. Presenting signs were flank pain or a palpable mass in most patients. Nine patients with lymphomas eventually developed disseminated disease. The tenth patient was alive and apparently free of disease 2 years after diagnosis, and another was living with an abdominal recurrence 3½ years after diagnosis. According to Farrow, there is only one report of a 5-year survivor of a primary renal lymphoma. The patient with the primary plasma cell tumor was also a long-term survivor.

Gonads

Gonadal involvement is infrequent in lymphomas of American patients and, when it does occur, is usually part of a generalized process. Testicular involvement is more frequent than ovarian (table 3).

Of lymphomas of American children in four series, involvement of ovaries was recorded in only 2 patients in the series of Jones and Klingberg (*84*) and in none of three other series (*72*, *85*, *86*). Testicular infiltration was recorded in 3 children, by Jones and Klingberg (*84*), by Sullivan (*86*), and by Dargeon [cited by Jones and Klingberg (*84*)].

Berg (*87*) found ovarian involvement in 3 of 13 necropsied female children 18 years and younger with disseminated lymphomas. Ages were 3, 13, and 18 years. Two were reticulum cell sarcomas and one a lymphosarcoma. Testicular involvement was found in 7 patients of ages 5 through 16 years. Two were reticulum cell sarcomas and the remainder were lymphosarcomas.

Ovarian involvement is quite prominent in African children with Burkitt's lymphoma and was observed in 7 of 12 female patients in the autopsy series of O'Conor (*12*). Ovarian lymphomas were also found at laparotomy of 4 patients who were investigated because of abdominal swelling. In some African patients, the ovarian masses were as large as 12 cm in diameter. Testicular involvement was the presenting complaint in 2 African patients and was recorded in 16 autopsies of male patients with Burkitt's tumors (*12*).

Woodruff *et al.* (*55*) reported 35 primary lymphomas of the ovary from the Ovarian Tumor Registry of the American Gynecologic Society. Three patients were younger than 10 years of age, 18 were between 21 and 40, and other patients were as old as 80 years. Four patients had a history of a lymphoma elsewhere in the body. Fifteen tumors were bilateral. The largest weighed 5280 g. Four patients were pregnant at the time of diagnosis. Fourteen tumors were classified as lymphocytic, 10 lymphoblastic, 10 reticulum cell sarcoma, and 1 giant follicular lymphoma.

All 6 ovarian lymphomas described by Nelson *et al.* (*51*) appeared to be part of generalized processes. Four were bilateral. Three were classified as lymphoblastic lymphomas. Ages of patients ranged from 19 years to 58; 4 patients were younger than 40 years.

Of interest to comparative oncologists is the frequency with which the ovaries are involved in Marek's disease of chickens (*45*). Both avian leukosis and murine lymphoma viruses are known to be transmitted to offspring via the ovum.

Uterus

The uterus may be involved in disseminated lymphomas but is rarely the site of primary lymphomas, and there are only 10 acceptable cases in the literature (*56*).

Migaki reports that prominent uterine infiltration occurs in approximately 10% of slaughtered cattle with lymphomas (*67*), a figure that might be even higher if this organ were examined "routinely." The uterus was not mentioned as the prominent site for lymphomatous infiltrates of other domestic animals (*67*, *83*).

Breast

Breasts are infrequent sites of primary or secondary lymphomas. Lymphosarcomas manifested as primary breast neoplasms 14 times in 25 years at Memorial Hospital in New York (*53*) and were 0.05% of all primary malignant neoplasms of the breast. Ages of patients ranged from 32–74 years. All tumors were unilateral. Several involved multiple sites within a single breast. Of seven 10-year survivals following various surgical procedures and irradiation, 3 patients subsequently developed recurrent disease. Four were alive and apparently free of disease 13, 15, 16, and 17 years after diagnosis.

Lawler and Richie (*60*) summarized information from 60 reported primary reticulum cell sarcomas of the breast. Tumors were bilateral in 2 patients. Ages of patients ranged from 9–75 years with the greatest frequency in the sixth decade. Two patients were children ages 9 and 12; the 9-year- old was a boy and the 12-year-old a girl. Of the adult patients, 3 were males. Only 4 patients survived 5 years; most had recurrences following therapy. Lymphomas of the breast are sometimes difficult to distinguish from anaplastic carcinomas.

One of the more bizarre phenomena of Burkitt's tumor syndrome is the propensity to involve the breasts of adolescent and mature females, particularly during the terminal months of pregnancy. This subject and its implications will be explored more thoroughly in a separate section of this paper.

Bone

Primary lymphomas of bone accounted for 11% of all malignant bone tumors (54 of 480) in the Memorial Hospital Tumor Registry (*78*). All were reticulum cell sarcomas. Thirty-nine percent of patients were female. Average age at diagnosis was 37.3 years, a decade higher than that for osteogenic sarcomas (26.5 years) and slightly lower than that for chondrosarcomas. Five- and 10-year survivals were nearly the same (31 and 28%) and were even higher (49% each) in Stage I disease.

McCormack and associates (*88*) reviewed the experience of the Mayo Clinic with 32 primary reticulum cell sarcomas of bone. Ages of patients ranged from 11 years to 52, and there was an excess of males (23 males to 9 females). Half of the patients were under 30. Eleven of 25 patients survived 5 years following amputation and irradiation. Most solitary tumors were located in bone of the extremities: 9 in femur, 3 tibia, 1 fibula, 5 humerus, 1 ulna, 2 scapula, 3 ileum, and 1 in a thoracic vertebra. Seven were "multicentric" involving more than one bone or an extra-osseous site. Histologically the tumors were similar to reticulum cell sarcomas elsewhere. Dahlin (*89*) reported that malignant lymphomas primary in bone accounted for 3.4% of bone tumors. He states that all types of malignant lymphomas can arise in bone. Most are reticulum cell sarcomas; lymphosarcomas and Hodgkin's disease are relatively less frequent.

Wang and Fleischli of the Massachusetts General Hospital (*63*) reviewed experience with 21 patients with primary reticulum cell sarcomas of bone. The two youngest patients were 15 years. The oldest was 75. Fifteen patients were under 40. Half were male and half female. In 16 patients the disease occurred in bones of extremities, 11 in femurs. Two male patients, ages 44 and 46, had primary involvement of the mandible. Eight patients survived 5 years following therapy by surgery and/or irradiation.

Other Sites

Unlike lymphomas of AKR and other strains of mice, primary lymphomas of the thymus are rarely recognized in man. If the thymus is involved in a generalized lymphoma, it is apparently usually obscured by intrathoracic masses of involved lymph nodes (*1*). An exception is the rare situation in which Hodgkin's disease originates as a primary lesion in the thymus.

Primary lymphomas of the spleen are difficult to recognize; the spleen is likely to be involved in early phases of disease in those patients whose initial signs include evidence of hypersplenism.

Liver, pancreas, and adrenal glands are frequently infiltrated by lymphomas as part of a generalized process, especially in the terminal phases in which few structures escape the process but are rarely, if ever, recognized as primary sites.

Epidemiologic Pathology of Hodgkin's Disease

Hodgkin's disease, a unique clinicopathologic entity, combines features of both inflammatory and neoplastic processes. Multinucleated giant cells of the Reed-Sternberg type are considered to be the malignant cells and are not only pathognomonic for the disease, but are essential for the diagnosis. Rappaport has provided precise descriptions and illustrations of Reed-Sternberg cells (*1*). Speculation concerning the origin and identity of Reed-Sternberg cells is a fascinating intellectual exercise and no completely satisfying explanation has been provided. These cells most nearly resemble bizarre histiocytes. Functions of the numerous inflammatory cells (eosinophils, lymphocytes, plasma cells, mast cells, histiocytes) are not known, but interesting theories suggest that they are a response of the host who attempts, usually unsuccessfully, to "combat" the neoplasm or its causative agent. Prognosis of the disease can be correlated to some extent with the numbers of infiltrating mature lymphocytes; those neoplasms with the fewest lymphocytes (lymphoid depletion) have the poorest prognosis, presumably because of an ineffective host reaction (*73*).

The immunologic deficiencies in patients with Hodgkin's disease are those of defective "cell-mediated" immunity and include decreased ability to develop delayed hypersensitivity to a variety of antigens and to reject homografts (*90*). The defects are apparently acquired and are not known to antedate onset of Hodgkin's disease. Capacity to form serum antibodies is not impaired. Patients with Hodgkin's disease are particularly susceptible to "opportunist" fungal diseases such as cryptococcosis.

The pathogenesis of Hodgkin's disease, if it is a single disease, is not clear, and the clinical and morphologic manifestations vary during different stages of disease and in different patients. The pathogenesis seems to involve a progression from a predominantly inflammatory to a predominantly neoplastic process. Not all stages of this theoretical sequence can be observed in a given patient and remissions, exacerbations, or death can occur during any stage. The debility of the patient may be out of proportion to apparent extent of disease and many patients are febrile.

Extent of disease indicated by clinical staging is a particularly useful tool in prognostication of Hodgkin's disease (*74, 75, 78*). As with other lymphomas, Hodgkin's disease may be initially localized in a single nodal or extranodal site, and surgical removal or radiotherapy during this stage has resulted in long-term survivals (table 2) (*78*).

Although Hodgkin's disease sometimes involves bone marrow, it does so irregularly and tends to form discrete "granulomatous" foci which do not characteristically replace normal elements. Leukocyte counts may be normal, leukopenic, lymphopenic, or leukemoid. Frank leukemias are rare, although at least three instances have been recorded of "Reed-Sternberg-cell-leukemia" (*91–93*) associated with circulation of typical Reed-Sternberg cells in peripheral blood. The leukocyte count of one patient was 140,000/mm^3, with 92% Reed-Sternberg cells. Bouroncle (*92*) reported that 37% of 135 patients with Hodgkin's disease had abnormal cells in peripheral blood and that in 18.5% of the patients circulating cells were typical Reed-Sternberg cells.

The epidemiology of Hodgkin's disease is fascinating and the continued accumulation of information concerning susceptible populations may provide insights into the nature of the disease. Hodgkin's disease has been observed in all areas of the world. In the United States, Hodgkin's disease causes death at an annual rate of 1.8/100,000, and accounts for 32% of all lymphomas (*4*). There is a predominance in males at all ages, a tendency noted in all types of lymphomas. Incidence occurs at 2 definite age peaks: one in the 15–34-year-old group and the other in patients past 45 years (*94, 95*). A similar age distribution has been recorded in the Netherlands, Denmark, and Sweden. Incidence rates for Negroes in the United States are lower than those in Caucasians. In Japan, incidences are lower than those of the United States in individuals younger than 40 years, but a steep rise in incidence similar to rates recorded in the United States occurs between ages 40 and 70 (*7*).

Cole, MacMahon, and Aisenberg (*95*) provided epidemiologic evidence that more than one etiologic event may be involved in causation of Hodgkin's disease. Their data emphasized the bimodal age curve observed in the United States. They reported an intriguing and unexplained geographic difference in incidence of the disease in younger patients in States of the northeastern United States compared to that of other sections of the country, a difference that did not occur in the older age group. The hypothesis is that geographic differences in incidence in younger individuals within the United States and elsewhere in the world suggest that more than one etiologic factor or event may be operative in different areas and at different ages.

HEMATOPOIETIC NEOPLASMS

Eddington and Maclean (*96*) found no differences in relative rates of Hodgkin's disease in several areas of west and central Africa. In Uganda, Hodgkin's disease accounted for 16% of all lymphoreticular neoplasms; one-fifth of all cases were in children (*15*). It must be emphasized that strict comparisons of relative incidences in Ugandan populations to those of the United States are not possible because of lack of census information in many parts of Africa. It is generally believed that the Ugandan population is younger than that of the United States, and this could alter relative ratios of different cell types.

Other reports have called attention to the unusually high rates of Hodgkin's disease in children of Peru (*97*) and Lebanon (*98*). Hodgkin's disease accounts for 16% of all malignancies in the Arab population of Zanzibar (*99*).

In the United States, Hodgkin's disease is rare in children (*4, 5*). Miller (*100, 101*) called attention to the abrupt rise in mortality rates from Hodgkin's disease in children that occurs near 11 years of age. Bailey *et al.* (*85*) reported an increase in numbers of childhood Hodgkin's disease beginning at 9 years of age. The increased susceptibility to Hodgkin's disease begins in late childhood and continues through adolescence and into young adulthood, particularly in males. Miller suggested that an explanation of the sudden upturn in incidence in late childhood may provide an "etiologic clue." One important event in immunologic development that terminates in late childhood, in the age range that marks the upturn in incidence of Hodgkin's disease, is "involution" of the thymus.

Precise counterparts of Hodgkin's disease have not been observed in animals except for rare "Hodgkin's-like" variants of histiocytic lymphomas occasionally observed in dogs (*70*) and in cattle (*102*). Giant cells with features of Reed-Sternberg cells have been described in lymphomas of both species (*70, 102*).

Burkitt's Tumor Syndrome

This bizarre and spectacular tumor syndrome is the most frequent neoplasm of children in areas of tropical Africa, such as Uganda and Nigeria. In parts of Uganda, incidence of the tumor averages 13.4/100,000 per year (*103*) and in Ibadan, Nigeria, it averages approximately 15/100,000 (*104*). Although similar tumors have been observed sporadically in other areas of the world, in no area has the incidence approached that of the regions of tropical Africa in which the tumor was originally described. The epidemiologic features suggest a unique environmental cause, possibly an insect-borne virus. The eponymic designation, Burkitt's tumor, honors the man whose insight and imagination sparked a new era in cancer epidemiology.

Morphologically, the neoplasm is considered to be a lymphoma (undifferentiated, Burkitt type) (*1*). Component cells are undifferentiated or "stem cells" interspersed with numerous clear histiocytes containing nuclear fragments. The term "starry sky" has been used for many years to describe a similar histiophagocytic phenomenon in germinal centers of active lymph nodes (*105*), and has been observed in a variety of nonneoplastic and neoplastic entities involving immunocytes (*106*). The importance of this single feature of Burkitt's tumor has been overemphasized and its presence erroneously considered by some to be pathognomonic for a Burkitt's tumor. This interpretation led to reports of Burkitt's lymphomas in man and animals from all areas of the world. Squire (*69*) described the lymphomas of animals which show features in common with Burkitt's lymphoma but emphasized that animal tumors should not be called Burkitt's tumors, a term which is by definition a human disease.

Wright, Bell, and Williams have recently reviewed the massive volume of information which has accumulated concerning Burkitt's tumor syndrome (*107*). They emphasized the spectacular involvement of the bones of the jaws of younger patients. This grotesque and unforgettable feature served as a useful index for identification of afflicted individuals and made the early epidemiologic studies in remote areas of Africa possible. The jaw tumor characteristically invades the tooth sockets, loosening or displacing the teeth (*108*, *109*), and invades exterior structures, such as adjacent muscle and joints. Loosening of the teeth is one of the common presenting features of the syndrome. Davies (*15*) described the early jaw lesions as multiple, small, osteolytic (by X ray) lesions beneath the lamina dura of developing tooth buds. The jaw tumors are unique in their capacity to attain monstrous proportions without lymph node involvement and without metastases. There has been no consistent opinion expressed concerning the precise site of origin of tumor cells in the mandible and maxilla, nor is it known whether the marrow cavity is involved primarily or secondarily. The tumor typically invades bone marrow cavities, yet leukemia is rare.

The lungs are rarely involved in even advanced cases. The pattern of spread is more suggestive of a multicentric origin than an initially localized process that later metastasizes, and is usually present in multiple areas of the body at the time of diagnosis in a pattern that is consistent with a generalized viral disease.

Sites of initial involvement or presentation of Burkitt's tumor are predominantly extralymphnodal (*15*). Of 197 non-nodal lymphosarcomas of African children, mostly Burkitt's tumors, almost half presented as enlargements of the jaws, particularly in younger patients. Fifty (one-fourth) presented as retroperitoneal or abdominal masses, and 27 (one-eighth) presented as ovarian tumors (*15*). In contrast, lymphomas of African adults of the same area and those of both children and adults of the United States and England usually originate in lymph nodes. Lymphomas of African adults resemble adult lymphomas in Britain; lymphosarcomas predominate and tend to be nodal in presentation (*15*). Lymphosarcomas comprise almost half of Ugandan adult lymphomas (193 of 417 total lymphomas); approximately one-fourth are reticulum cell sarcomas (124 of 417) and one-fourth Hodgkin's disease (100 of 417), figures which are slightly different than those of the United States. The Ugandan population is younger than that of the United States, and age differences alone can significantly affect relative incidences of cell types.

Some fascinating aspects of Burkitt's tumor syndrome are the spectacular responses to doses of chemotherapeutic agents (*107*, *110*) and the tendency of the tumor to remain localized for long periods of time and to grow to enormous sizes without involving regional lymph nodes. These phenomena have suggested that unusual immunologic factors involved in the pathogenesis of these tumors serve to retain them in initial sites for relatively long periods of time and to assist in the rapid regression following chemotherapy. A possible "priming" or "stimulating" effect on the reticuloendothelial system by prior or concomitant parasitic infections, as malaria, which affects a large percentage of the population in areas "endemic" for Burkitt's tumor, has been suggested as a special situation that predisposes children living in these areas to reticular malignancies (*110*).

Burkitt's tumors are rare in adults. When they do occur in adults, they do so in two rather unique situations: 1) women of childbearing ages, particularly during pregnancy (*111*), and 2) individuals who immigrated to endemic areas during adolescence or early adulthood (*107*). The latter observation suggests that early childhood experience with an environmental factor peculiar to the area provides protection from the tumor during adulthood. Epidemiologists have also noted a "shift" in geographic areas of tumor prevalence (*107*).

A bizarre phenomenon is the apparent unusual susceptibility of adolescent and adult women to Burkitt's tumors during the latter months of pregnancy. Bilateral Burkitt's tumors developed in breasts during pregnancy or lactation in six reported cases (*111*, *112*). Ages ranged from 15–36, higher than those of most patients with Burkitt's tumors. One patient who refused treatment was reported, unofficially, to have undergone a spontaneous remission. The other five patients succumbed to generalized disease despite chemotherapy. Postmortem examinations revealed that one patient had involvement of the ovaries in addition to bilateral breast disease; uterus, pituitary, and brain were also involved in a second patient. Breast tumors were characteristically multiple discrete masses and were not attached to skin or muscle. No patient had involvement of axillary nodes despite the massive sizes that the tumors attained. Treatment was delayed in all patients because the tumors developed during pregnancy and caused diffuse enlargements rather than localized masses. Neoplasms were not suspected until terminal stages in five patients.

Breast involvement as part of generalized Burkitt's tumors has also been described in three patients who were apparently not pregnant at the time of onset (*111*, *113*). A 23-year-old woman, who was not reported to be pregnant, succumbed to widespread Burkitt's tumors which included massive bilateral involvement of breast (*111*). Two girls (12 and 14 years) developed unilateral breast tumors following surgical removal of Burkitt's tumors of ovaries and chemotherapy (*113*). Survivals following surgical removal of involved breasts were 2 and 2½ years at the time of the report. Breast involvement by a Burkitt's tumor was reported by Kovi and Laing (*114*) in a 15-year-old girl but few details were recorded. A dramatic decrease in size of a breast tumor was observed in one patient following chemotherapy (*113*).

Shepherd and Wright (*111*) found in the world literature seven reported cases of bilateral breast lymphosarcomas associated with pregnancy. Two cases of bilateral lymphosarcomas of the breast associated with bilateral ovarian lymphosarcomas have also been recorded.

It is tempting to explain these phenomena as peculiar alterations of the immune apparatus that occurs during pregnancy associated with development of tolerance to the "foreign" antigens of the fetus.

Attempts to define the "constitutionally susceptible" individuals in endemic areas of Burkitt's tumor have involved exploration of genetically controlled features, such as blood groups and hemoglobin types (*115*). No significant differences were found in the ABO blood group systems in Burkitt's tumor patients and normal controls. Electrophoretic analysis of hemoglobins from patients and controls indicated an excess of the AA genotype among patients with Burkitt's tumor compared to those of controls. A large proportion of controls were homozygous (SS) or heterozygous (SA or SC) for the sickling trait. The S or "sickling" trait may be present in as many as one child in twenty-five in some areas of Africa, and possession of this trait is known to confer partial protection against the lethal effects of falciparum malaria. The AA genotype is also associated with susceptibility to malaria. Burkitt's tumor has been observed in one child with SC hemoglobin and in another with sickle-cell anemia, indicating that the S factor does not confer absolute protection against Burkitt's tumor. Burkitt's tumor has also been observed in children of the AC genotype.

Chromosome abnormalities in Burkitt's tumor cells, as with cells of most lymphomas, have been inconsistent (*107*).

Other Lymphomas of Children

Burkitt's tumor focused attention on lymphomas of children elsewhere in the world. In the United States, lymphomas of children differ from those of adults in several respects. Compared to adult lymphomas, childhood lymphomas are more likely to be present in extralymphnodal sites. The spectrum of extranodal primary sites is somewhat different. "Leukemic transformation" is more likely to occur in children.

Lymphomas occur much less frequently than *primary leukemias* in American children under 15 years of age, and account for fewer than

10% of all childhood hematopoietic neoplasms (*4, 5*). This figure is only approximate because of discrepancies in final diagnoses of patients in whom lymphosarcoma undergoes "leukemic transformation." In contrast to the situation in the United States, lymphomas (Burkitt's type) predominate in certain areas of tropical Africa, and leukemias are comparatively infrequent (*15*). Because of the low incidence, large series of lymphomas of American children are difficult to obtain and reports of 5–30 years' experience in 4 major medical centers in the United States provided descriptions of only 217 (*72, 84–86*). In all patients, leukemia was excluded at the time of diagnosis by examination of peripheral blood and in some cases, bone marrow, although "leukemic transformation" occurred eventually during the course of disease in from 10–40% of the children with lymphomas, depending on the criteria for this diagnosis.

Data from the 4 reports could not be strictly compared because of differences in criteria for classifying these tumors. The major discrepancies in classification occurred with forms intermediate between those that are clearly lymphocytic and those composed of reticulum cells. The terms *lymphosarcoma* and *lymphoma* were used synonymously and included *reticulum cell sarcomas* in 3 series. Of 69 patients in the New York Memorial Cancer Hospital series (*72*), 61% were classified as having lymphosarcomas, and the remainder as having reticulum cell sarcomas. Of the 43 patients of the St. Louis series (*84*), 88% had lymphosarcomas, and the remainder had reticulum cell sarcomas. Only 2 of the 29 lymphomas of the Texas series (*86*) were specifically designated reticulum cell sarcoma; the remainder were called lymphocytic lymphosarcomas, lymphoblastic lymphosarcomas, or simply malignant lymphomas. No reticulum cell sarcomas were included in the Mayo Clinic series (*85*). A diagnosis of Hodgkin's disease was made in one-third of 76 lymphomas of the Mayo Clinic series (*85*) but was specifically excluded from the other 3 series. It is possible that a disproportionately large number of children with Hodgkin's disease, which is rare in children compared to adults, would receive diagnosis and therapy at the Mayo Clinic.

A predominance of male children observed in all 4 series was in the order of 2 or 3 males to 1 female.

Ages of children with lymphomas in 7 series compiled by Jones and Klingberg (*84*) indicated 2 peaks, one at 3–4 years and a second at 11 years. The early 3–4-year peak was the same as that for childhood leukemia in the United States (*4, 5*) and Denmark (*29*). Wright's analysis of 50 English childhood lymphosarcomas revealed only the early 3–4-year age peak (*66*). These differences may only reflect the types of diseases included in each series and, because incidence of Hodgkin's disease rises sharply in late childhood (*100*), inclusion of this disease in a series would have the effect of contributing a secondary peak in late childhood. The peak age of Burkitt's lymphoma at 5–7 years (*11*) is slightly older than for lymphomas and leukemias of American, Danish, and English children (*4, 5, 29, 66*).

Lymphomas are rare in the neonatal period. Only 1 of 221 childhood lymphomas compiled by Jones and Klingberg (*84*) was in a child younger than 1 year. Charache (*116*) described an infant in whom a lymphosarcoma of skin of a shoulder was reported to be present at birth. Following surgical excision and irradiation, there was no evidence of recurrence when the infant died several months later of an infection. Only 1 of Wright's (*66*) English lymphoma patients was younger than 1 year. In the United States, 6 deaths in each of 1964 (*4*) and 1965 (*5*) of children under 1 year of age were attributed to reticulum cell sarcomas or lymphosarcomas. In 1964, only 1 child under a year reportedly died of Hodgkin's disease (*4*), and none were recorded in 1965 (*5*). In contrast, leukemias caused deaths in 86 (1964) and 83 (1965) children under 1 year (*4*, *5*).

Rosenberg *et al.* (*72*) compared sites of involvement of lymphomas in 69 American children with those of 1,200 adults and noted the following differences: Peripheral nodes appeared to be involved initially in 43% of children with lymphosarcomas and in 65% of adults. Twenty-three percent of children appeared to have primary involvement of abdominal structures and nodes compared to 12% of adults. (It is difficult to accurately determine "primary" sites and true extent of disease at the time of diagnosis; these figures indicate the sites that most likely caused initial symptoms or signs or were unusually prominent at the time of onset.) Nine percent of childhood lymphosarcomas involved intestines primarily, while only 0.8% of adult tumors manifested as primary intestinal tumors. Adult lymphosarcomas were primary in stomach in 2.5% of patients, while none of the childhood tumors of the Memorial Hospital series manifested as primary gastric tumors. Two children of the Mayo Clinic series had primary stomach tumors (*85*). Eight percent of childhood tumors presented as mediastinal masses, and several had evidence of superior vena cava obstruction, while only 3% of adult tumors appeared to be primary in the mediastinum. Of particular interest in comparisons to Burkitt's tumors in Africa, in which primary involvement of bone particularly of the jaws is one of the hallmarks of the disease, 13.8% of lymphosarcomas of American children of the Memorial Hospital series were primary in bone and 2.4% of those of adults. Most primary bone lymphomas are reticulum cell sarcomas and are described more completely in a separate section of this paper. Involvement of the jaws was not mentioned in the Memorial Hospital series, but the maxilla was involved in one of Sullivan's patients.

Jones and Klingberg (*84*) compiled alleged "primary tumor sites" of 144 childhood lymphosarcomas reported in the literature. Most frequent primary sites were : peripheral lymph nodes, usually cervical, 30%; abdominal viscera and/or nodes, 22%; mediastinum, 15%; pharynx, 7%; and bone, 9%. Smaller numbers were initially observed in miscellaneous sites, such as skin, prostate, testis, breast, eye, and central nervous system. A composite of the 4 series reviewed also includes kidneys, maxilla, tonsils, and ovaries as rare primary sites of childhood lymphomas. Such figures are not completely reliable because it is not possible to determine precise extent of disease at the time of diagnosis. However, sites listed were those that first called attention to the presence of disease and indicated dominant sites. Most patients of American series have peripheral lymph node involvement at time of diagnosis, in contrast to Burkitt's lymphomas of African children in which extranodal sites predominate and involvement of peripheral nodes is unusual.

Lymphocytic types of lymphomas predominate in American children and were recorded in 82% of the series of Jones and Klingberg. The remainder were reticulum cell sarcomas. Hodgkin's disease and giant follicular lymphomas are rare in children and were excluded from this series.

Reported rates of leukemic transformations of lymphomas vary with age. In the review of 43 cases of childhood lymphosarcoma by Jones and Klingberg in 1963 (*84*), leukemia was present in 30%; in contrast, for adult patients, the rates of leukemic transformation were only 7%, according to Rosenberg *et al.* (*72*). Wright described "leukemic transformation" in 10 of 50 English children with lymphosarcoma (*66*). Leukemic transformation even as a terminal event is rare in African children with Burkitt's lymphomas.

DISORDERS OF THE IMMUNE-PROTECTIVE SYSTEM

Disorders of the immune-protective system contribute to the increased susceptibility of patients with hematoneoplastic diseases to infections by viral, bacterial, fungal, and protozoal agents. Decreased numbers of functional blood granulocytes, decreased or otherwise abnormal capacity to produce immunoglobulins, or defective cell-mediated immune reactivity may contribute to the susceptibility of a patient. Administration of cytocidal or immunosuppressive drugs may contribute to the defects.

Immune defects may antedate onset of frank neoplasia in some patients, particularly "autoimmune" disorders and dysglobulinemias (*3*). An increased susceptibility to lymphoreticular disease has been noted in patients with Sjögren's syndrome, a disease with prominent autoimmune features (*117*).

Miller (*118*) summarized immunologic defects of patients with leukemias and lymphomas. Abnormalities in formation of gamma globulins were observed most frequently in patients with lymphoproliferative diseases, particularly chronic lymphocytic leukemia. Autoimmune hemolytic anemias were observed most frequently in chronic lymphocytic leukemia and lymphosarcomas. Infectious diseases complicated chronic lymphocytic leukemias and acute leukemias more frequently than other neoplasms. One-third of Miller's patients with chronic granulocytic leukemia had *hypoglobulinemias*. The highest incidence of cryptococcosis and tuberculosis occurred in patients with Hodgkin's disease.

Krauss and Sokal (*119*) reported abnormal serum globulins in 9 patients with lymphomas. Seven patients had small lymphocytic neoplasms, 6 lymphosarcomas, and 1 chronic lymphocytic leukemia. One patient had a mixed lymphocytic-histiocytic type and another had Hodgkin's disease. There was little correlation between type of paraprotein and histologic appearance of the tumor. Appearance of a paraprotein during the course of disease was associated with an unfavorable clinical course.

Paraproteins (abnormal serum immunoglobulins) occur most characteristically in plasma cell myelomas, Waldenström's macroglobulinemia, and other dysglobulinemic syndromes. In addition to their occasional appearance in patients with lymphocytic leukemias and lymphocytic lymphomas, paraproteins have been reported in a patient with chronic myelogenous leukemia (*120*) and in a child with acute leukemia (*121*).

Patients with Burkitt's lymphomas usually have normal serum globulins, although some may have lower than normal levels of IgM (*107*).

Kyle *et al.* (*37*) reported acquired hemolytic anemias complicating leukemias and lymphomas in 27 patients. Sixteen were chronic lymphocytic leukemias, 7 Hodgkin's disease, 3 follicular lymphomas, and 1 lymphosarcoma. All patients were past 30 and most were over 50 years of age. Hemolytic anemia was the initial symptom in some patients with *lymphomas*, while it tended to be a secondary event in *leukemia*. Positive Coombs' tests were observed in approximately half of the 27 patients, both leukemias and lymphomas. Four of 8 patients improved following splenectomy.

Control of infections is a formidable problem in patients with hematoneoplastic diseases. Although many patients with leukemias and lymphomas can successfully overcome viral infections, Herpes zoster and generalized infection with cytomegalovirus are troublesome complications of these diseases. A wide range of bacteria infect patients with defective granulopoiesis. The susceptibility of patients with Hodgkin's disease to cryptococcosis has already been mentioned, and patients with other lymphomas and chronic lymphocytic leukemia are also unusually susceptible to fungal diseases (*122*). The increased susceptibility of patients with lymphoreticular diseases to protozoal disease, such as toxoplasmosis, is interesting. Of 20 cases of toxoplasmosis in patients with neoplastic diseases reported by Vietzke and associates (*123*), 8 had Hodgkin's disease, 2 reticulum cell sarcomas, 1 multiple myeloma, and 6 had leukemias of various cell types including chronic lymphocytic leukemia. An additional patient had myeloid metaplasia. The 2 nonhematopoietic neoplasms were a melanoma and an ovarian carcinoma. Ages of patients ranged from 6–76 years. Most clinical evidence indicated involvement of the central nervous system; several patients had a syndrome of skin rash, myocarditis, pneumonitis, and/or lymphadenopathy.

RELATIONS TO NEOPLASMS OF OTHER SYSTEMS

Berg (*124*) reported that only cancers of the skin occur frequently enough in patients with leukemia or lymphomas to suggest an association; a significantly increased risk for other nonhematopoietic cancers was not found. Factors involved in this increased risk situation were not identified. In descending order, the increased risks of second cancers were in patients with myeloid leukemias, lymphocytic leukemias, and all types of lymphomas. There were too few myelomas to provide significant statistics. Patients with nonhematopoietic neoplasms did not develop leukemias or lymphomas at significantly higher rates than the control population (*87*).

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Primary Lymphomas of the Human Gastrointestinal Tract

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LYMPHOMA CELLS in the gastrointestinal tract are common as part of generalized disease. In addition, however, some lymphomas appear to arise as a local disease of the stomach or intestine, and their subsequent behavior can support this assumption. Primary gastrointestinal lymphomas are sufficiently frequent that every pathologist and every surgeon working in the abdomen will find an occasional case; yet, at the same time, they are sufficiently rare so that it is difficult to assemble a series large enough to include even the major variations in pathology and clinical course. Table 1 illustrates this point. One in 20 patients with lymphomas in the series of Rosenberg *et al.* had primary gastrointestinal involvement (*1*); in contrast, only 1 in 100 intestinal tumors were lymphomas in the series of Warren and Lulenski (*2*). Frequency ranged from 20% of tumors of the small intestine to three-tenths of 1% of colon and rectal tumors (*2–4*).

TABLE 1.—Frequency of occurrence of primary gastrointestinal lymphomas

	Author and reference
4.5% of 1,269 lymphomas, all sites	Rosenberg *et al.* (*1*)
1.0% of 3,132 gastrointestinal tumors	Warren and Lulenski (*2*)
2.5% of 1,685 gastric tumors	McNeer and Berg (*3*)
20.0% of 167 small intestinal tumors	Faulkner and Dockerty (*4*)
0.3% of 2,510 colon and rectal tumors	Warren and Lulenski (*2*)

Table 2 shows the comparative rates of occurrence of primary lymphomas in the main sections of the gastrointestinal tract as reported in the literature. Approximately half of gastrointestinal lymphomas were gastric, and half intestinal. Esophageal lymphomas were extremely rare, about a fraction of 1%. Table 3 is a composite tabulation from those series that further subdivided intestinal lymphomas. More than half of intestinal lymphomas occurred in the ileocecal region. At the same time, despite the fact that appendixes contain so much lymphoid tissue, few series listed primary appendiceal lymphomas. Some lymphomas probably do arise in the appendix but do not become symptomatic until they overgrow into the cecal region. Wychulis, Beahrs, and Woolner (*5*) reported 1 appendiceal lymphoma in 69 patients with malignant lymphomas of the colon, 39 of which were located in the cecal or ileocecal region. Another point of interest is the fairly low frequency of rectal lymphomas. Reported rates were higher before Helwig and Hansen outlined the basis for separation of benign lymphoid polyps (*6*).

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Table 2.—Distribution of primary gastrointestinal lymphomas: composite series of 362 cases

Lymphomas	Percent
Stomach	51
Small intestine	33
Large intestine	16

Table 3.—Distribution of intestinal lymphomas: composite of detailed series of 177 cases

Lymphomas	Percent
Duodenum	6
Jejunum	16
Ileum	40
Cecum	18
Colon	8
Rectum	12

Patients with lymphomas of the stomach and rectum are about 10 years older on the average than the totality of all lymphoma patients. The average age of about 60 for these patients is the same as that for hospital patients with rectal and stomach cancers. Lymphomas of the small intestine and colon occur with a bimodal distribution similar to that of lymphomas originating in lymph nodes. There is a peak below the age of 10 years, in addition to an adult age distribution with an average age of 53. Primary lymphomas of the stomach are rare in children, even though they are the most common primary gastrointestinal tumors of childhood and secondary gastric involvement in lymphoma and leukemia is frequent. Only 2 children with gastric lymphomas are described in the literature (*7*). Apparently the distribution of lymphomas in the gastrointestinal tract at any age parallels that of lymphoid tissue. While lymphoid tissue is prominent in intestines of children and less conspicuous in adults, it appears in the stomach only as a sign of chronic gastritis, a disease of adult life.

GROSS PATHOLOGY

While lymphomas do not look like carcinomas, some anaplastic reticulum cell sarcomas are sources of confusion, since they present as bulky, fairly well-delineated masses. These can be grossly ulcerated centrally with firm, indurated borders and base. About a third of gastrointestinal lymphomas might be mistaken grossly for carcinomas. In the other two-thirds, the growth pattern is influenced by the tendency of better differentiated lymphomas not to destroy muscle. Instead, the tumor stops abruptly at the muscle border. When this characteristic is dominant, as it is in about half the cases, there will be diffuse thickening with apparent mucosal hypertrophy in the stomach, or a dilated "garden-hose" segment of intestine. This type of induration is not stenosing; when obstruction is the presenting symptom, it arises from intussusception or other block by the third type of tumor growth, exophytic, a polypoid protrusion into the lumen, again with little growth the other way through muscle.

Almost all gastrointestinal lymphomas removed at surgery are ulcerated. Presumably, with the tumor immediately beneath the epithelial layer, a break easily occurs but is difficult to repair. Despite this, neither symptoms of ulcer nor bleeding are a common complaint in lymphoma. With gastric lymphomas, there usually are discomfort and dull pain unrelated to food; intestinal lymphomas are most often discovered because of an abdominal mass or because of intestinal obstruction. Pain and bleeding are uncommon.

Growth and spread of primary gastrointestinal tumors occur by three modes.

1) Separate foci of lymphoma tend to occur in areas of the mucosa away from the main tumor. We had evidence of multicentricity in 60% of primary gastric lymphomas (*3*). The multicentricity of origin and the tendency to lateral unobtrusive spread of the primary tumor are the reasons that involvement of margins of resected specimens is reported in up to 70% of gastric lymphomas, as reported by Burnett and Herbert (*8*). Because of the tendency of lateral spread and multicentric origins, postoperative radiation is recommended for this disease.

2) The tumor extends directly into other structures; this extension occurs most often with the more anaplastic tumors. Extension through the wall accounts for the rates of 14% perforation of gastric lymphomas found by Friedman (*9*) and of 36% extension into other structures found in our series (*3*). Tumors primary in the stomach involved the pancreas, spleen, and liver. Those of the intestine most often involved mesentery, but sometimes an additional segment of bowel or urinary bladder was invaded which resulted in the formation of fistulas. The tendency to extension is important, because it accounts for the fact that, at least half the time, extended procedures are necessary for treatment. This in turn accounts

for the high postoperative mortality which, until recently, has been approximately 20%.

3) The tumor spreads by lymph nodes. When only a few nodes are involved, they are the same regional nodes that would be expected to be involved with a carcinoma of the same site. Nodes were involved in 84% of our stomach cases. Friedman's rates for those of stomach, about 70% (*9*), are lower than those of our series (*3*) and nearly the same as those reported by Faulkner and Dockerty for lymph nodal involvement with primary small intestinal lymphomas (*4*). Even Faulkner and Dockerty's figure indicates that many of these neoplasms are in relatively late stages by the time of resection. It should also be emphasized that fewer than half of the *enlarged* nodes in our specimens showed tumor. The rest were only hyperplastic. When nodes were involved, the involvement could be partial and would have the pattern of a true metastasis, with the normal node sharply separated from the sarcoma (fig. 1).

HISTOLOGIC FEATURES

Gastrointestinal lymphomas range from well-differentiated lymphocytomas to anaplastic reticulum cell sarcomas (table 4). The most frequent cell type unfortunately is midway between these two extremes (fig. 2). These intermediate types are sometimes grouped with the large-cell tumors and sometimes with the small-cell tumors. For example, Friedman (*9*) found only 15% of gastric lymphomas to be reticulum cell sarcomas. At the other extreme, we found 86% (*3*). Most other series lie somewhere nearer 50%. Our approach was an extrapolation based on our experience with generalized lymphomas; the so-called "lymphoblastomas" or intermediate-sized cell tumors behave as aggressively as large reticulum cell sarcomas, so that only the truly small-cell lymphocytomas have a significantly better prognosis. In the stomach, only the lymphocytic group had significantly fewer metastases to nodes, no organ invasion, and a significantly better prognosis. Stobbe, Dockerty, and Bernatz (*10*) reported that survival of patients with primary gastric lymphomas correlated with both extent of disease and maturity of cells.

TABLE 4.—Relation of cell type to presentation and behavior of primary gastric lymphomas (*3*)

Cell type	Number of cases	Involvement of nodes (%)	Invasion of other organs (%)	3-year survival
Lymphocyte	5	40	0	4/4
Intermediate	19	84	37	2/8
Reticulum cell	12	100	50	3/6

Another confusing problem is the question of the true incidence of primary Hodgkin's disease of the gastrointestinal tract. Gall and Mallory's series of 70 cases (*11*) did not include a single example. On the other hand, other series have reported from 10–20%. This discrepancy is difficult to explain, but may result from the fact that features usually considered to be characteristic of Hodgkin's disease can be found in *all types* of gastrointestinal lymphomas. For example, eosinophils are a normal component of the mucosal lymphoid complex, especially that of the stomach. The more lymphoid cells there are in infiltrates of any kind, whether malignant or benign, the more eosinophils one may expect. They are frequent in subacute ulcers, chronic gastritis, benign lymphoid hyperplasia, and carcinoma as well as in lymphomas. *Fibrosis* is also frequent in gastrointestinal lymphomas. Figure 3 shows a characteristic pattern, but a more "Hodgkin's-like" variety (fig. 4) also occurs. Perhaps most important is the peculiar tendency of gastrointestinal reticulum cell sarcomas to contain large multinucleated giant cells (fig. 5). There is some resemblance to Reed-Sternberg cells, but there are also differences. Rather than trying to divide giant cells with precision, an easier way seems to be examination of the other malignant cells. A population of medium-size malignant reticulum cells is not characteristic of Hodgkin's disease. Instead they are either more benign-looking lymphocytes and plasma cells or the very anaplastic cells of Hodgkin's sarcoma. In our experience, this type of reticulum cell sarcoma is found most often in the gastrointestinal tract, although it is also seen elsewhere in the abdomen and occasionally in tumors apparently beginning in peripheral nodes.

Lymphocytic lymphomas and benign lymphoic hyperplasias are more difficult to differentiate. This problem is more likely to occur in stomach and rectum than elsewhere in the gastrointestinal tract; in the small intestine and colon, hyperplasias, likely to remain asymptomatic, will not be discovered. On the other hand, in the stomach, hyperplasia can produce digestive disturbances; in the rectum, hyperplastic lymphoid polyps may produce partial obstruction, bleed, or simply be found on routine physical examination. The gross appearance often assists in differentiation. There is a limit to the size of a benign mass, and in the rectum pure polyps almost always are benign while the flatter, indurated, bulky masses can be expected to be true lymphomas. Microscopically, benign masses will have some residue of normal follicular structure (*12*). Structures in lymphomas that resemble follicles under low power prove to be otherwise under high magnification (fig. 6). The other basis for separation is fine cellular detail. With a questionable lesion, irregularity and hyperchromicity of the nuclei rule out a benign lesion.

CONCLUSIONS

Lymphomas can be primary in the gastrointestinal tract in exactly the same way that carcinomas or leiomyosarcomas are primary. The tendency to mucosal and submucosal growth (fig. 7), plus the foci of early change in separate mucosal nodules, leaves little doubt that these lymphomas arise in the gut wall and do not invade it from adjacent nodes. It does not seem farfetched to equate the small separate foci with the small separate foci of carcinoma *in situ*, sometimes found in the epithelium around invasive cancer. The parallel is especially interesting, since similar early foci were *not* found in lymph nodes in our material. Rather, as was mentioned, the node lymphoma looked like metastases with either partial or complete replacement by definite tumor.

Perhaps most important is the life history of the treated disease. For primary gastric lymphomas, published figures suggest that half the patients who are operable and survive the postoperative period can expect to remain free of disease thereafter. Even for intestinal lymphomas, coming as late as they do for operation, the expected cure rate is about 20%. This is true for both children and adults.

If for these reasons we accept the concept of primary gastrointestinal lymphomas as localized, reasonably curable cancers, it becomes easy to look beyond and to speculate how many lymphomas of other sites also begin as localized tumors. For instance, how many lymphomas beginning in nodes might at one stage be fit subjects not just for palliation but for cure? More important than any argument about cell type and any effort to make our diagnostic criteria a little sharper, the clarification of this last point stands today as the major problem for clinicopathologic attack.

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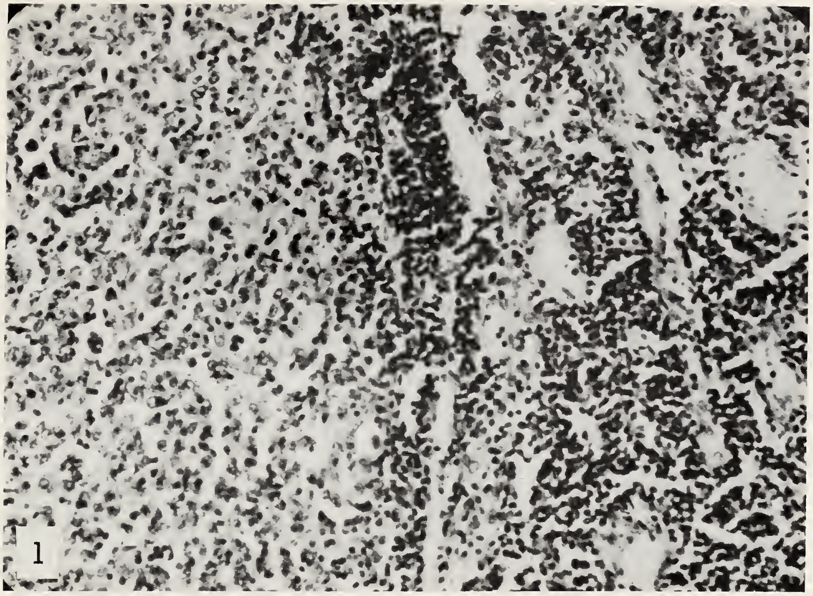


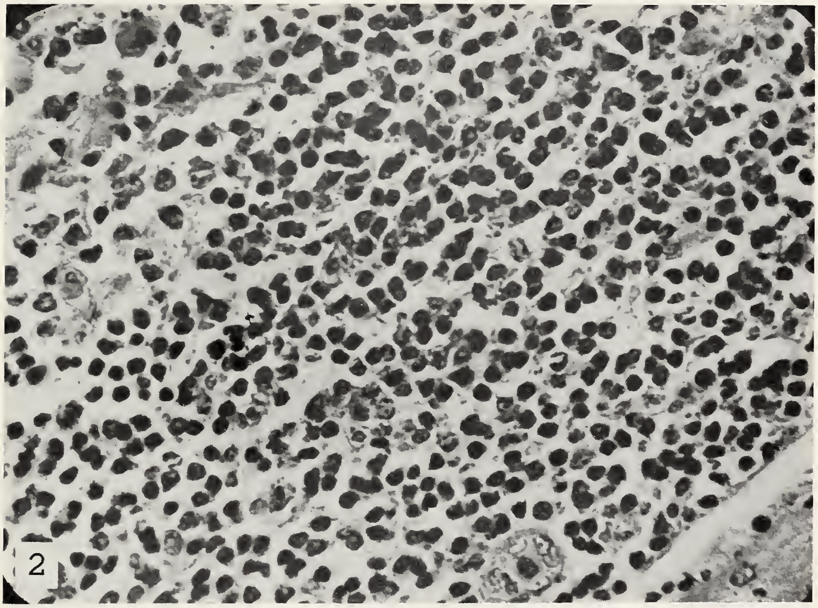
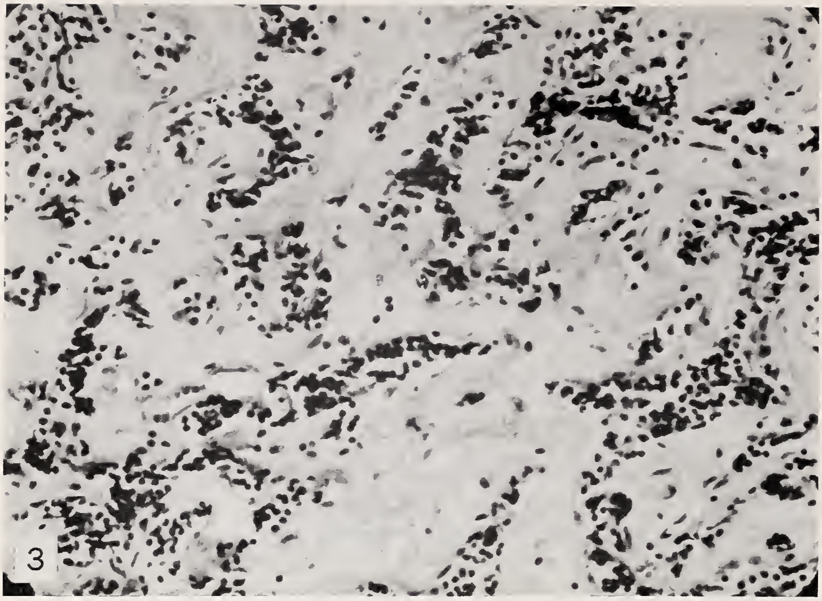


FIGURE 1.—Partial involvement of a lymph node by "metastatic" lymphoma, showing sharp separation of neoplasm (*left*) from noninvolved portion of node (*right*).

FIGURE 2.—Primary gastrointestinal lymphoma of the "intermediate" cell type. Cells are larger and more pleomorphic than mature lymphocytes.



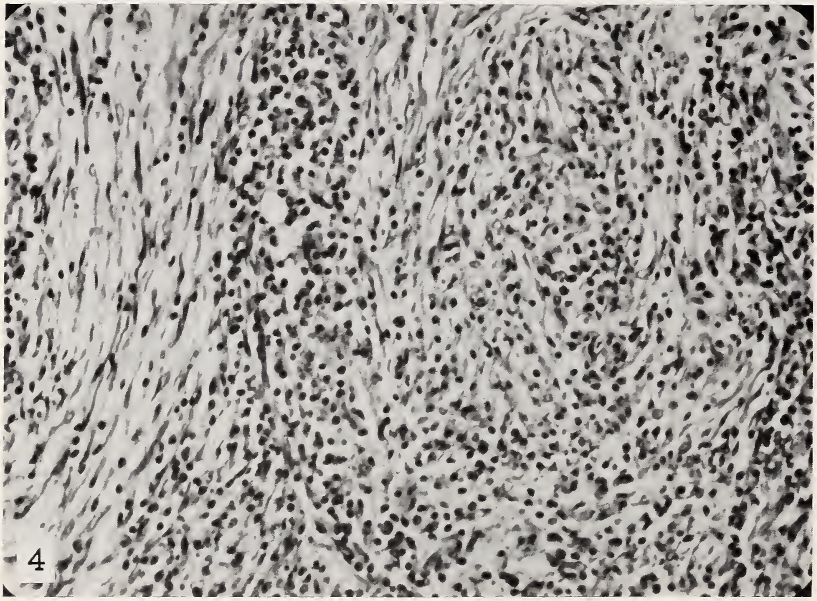
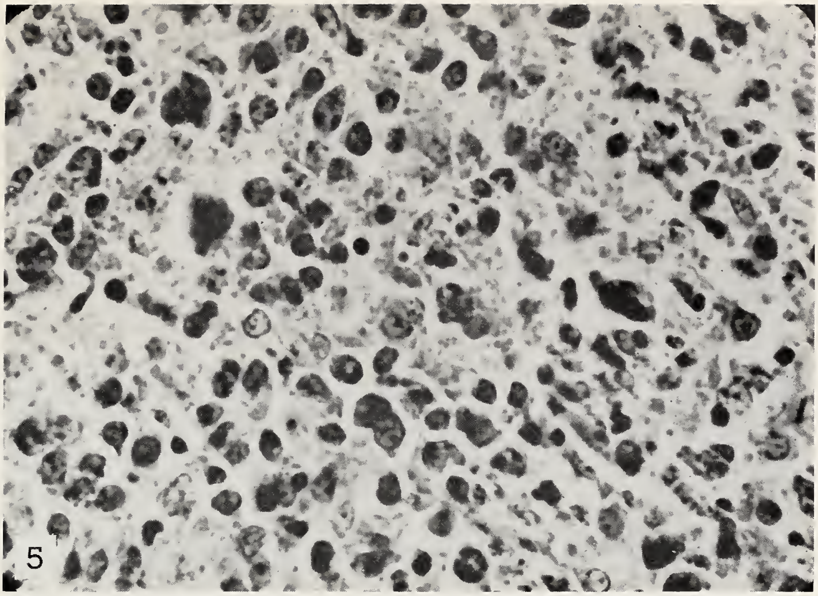


FIGURE 3.—Primary gastrointestinal lymphoma with prominent collagen.

FIGURE 4.—Nodular gastrointestinal lymphoma with diffuse fibrosis.



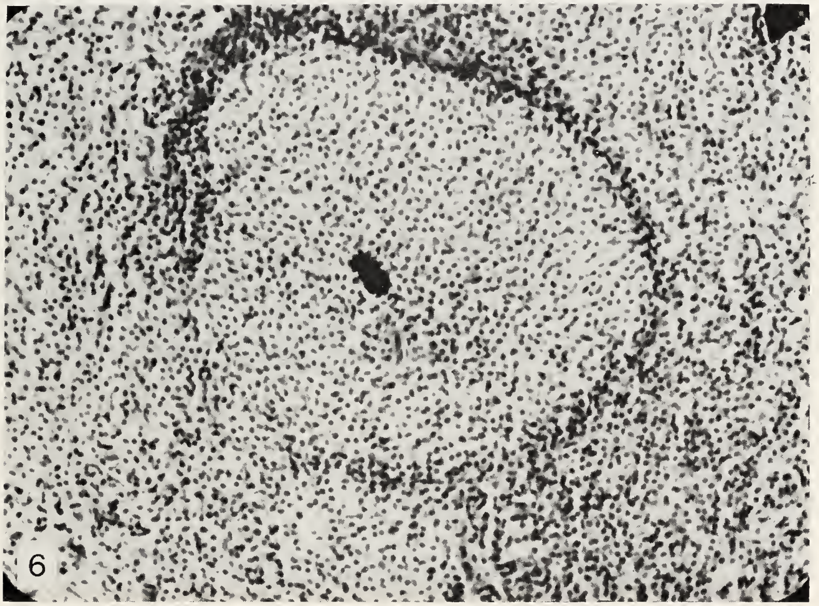


FIGURE 5.—Reticulum cell sarcoma, primary in the gastrointestinal tract. Multinucleated giant cells resemble Reed-Sternberg cells. Other features of Hodgkin's disease are absent.

FIGURE 6.—Primary gastrointestinal lymphoma with a nodular pattern. Cells inside the "pseudofollicle" are the same as those outside.

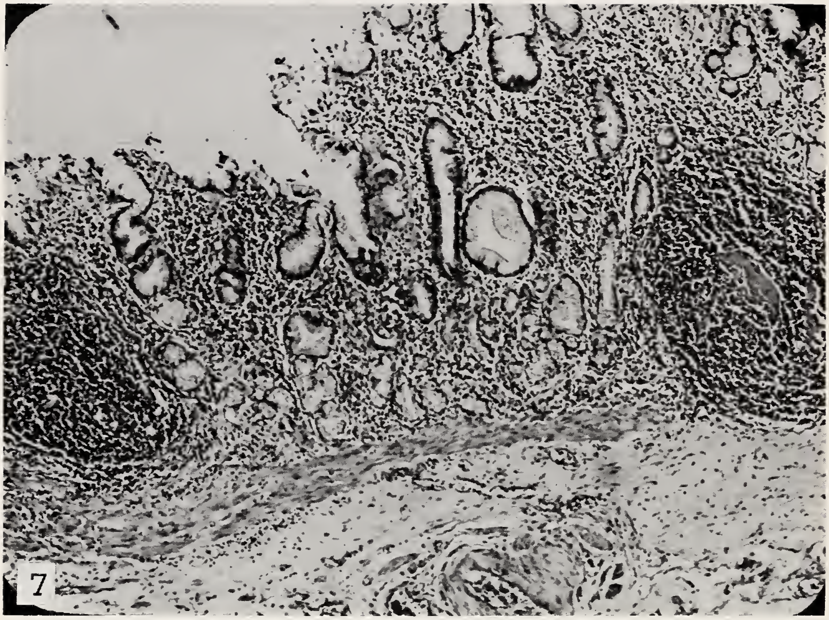


Figure 7.—Lymphoma originating in mucosa of the stomach.

Constitutional Disorders of Man Predisposing to Leukemia and Lymphoma[1]

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SUMMARY

Accumulating evidence suggests that certain constitutional diseases associated with cytogenetic defects (mongolism, the syndromes of Bloom and Fanconi, and possibly Klinefelter's syndrome and D-trisomy) carry an increased risk of leukemia, while congenital disorders of immune deficiency and susceptibility to infection (ataxia-telangiectasia, Wiskott-Aldrich syndrome, agammaglobulinemia, and Chediak-Higashi syndrome) predispose to the lymphomas. These syndromes and a variety of other observations implicate chromosomal aberrations, immunologic deficiency, and genetic factors in the origin of leukemia and lymphoma. There are significant advantages to the use of constitutional disorders and other "high-risk" conditions in laboratory and epidemiologic research on these neoplasms.—Nat Cancer Inst Monogr 32: 221–232, 1969.

IN recent years there has been an increasing recognition that certain cancers of childhood are associated with specific congenital defects (*1*). These relationships suggest that a better understanding of pathogenic mechanisms and aberrations underlying these defects would benefit research into the origins of the associated neoplasms. The purpose of this presentation is to review the congenital disorders which predispose to leukemia and lymphoma and to discuss the opportunity these disorders provide for identifying etiologic factors in hematopoietic neoplasia.

CONGENITAL CYTOGENETIC DISORDERS

Most congenital or constitutional disorders linked especially to leukemia have chromosomal aberrations of one sort or another (*1*, *2*).

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[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Mongolism

The earliest and most widely studied of the constitutional disorders is mongolism or Down's syndrome. Among its many manifestations are the characteristic facies (including epicanthal folds and an enlarged protruding tongue), a single transverse (simian) palmar crease, mental retardation, small stature, and occasionally congenital heart disease. The characteristic chromosomal abnormality (G-trisomy, an extra #21 chromosome) appears to be due to nondisjunction, with an error in meiosis probably occurring before conception or possibly soon afterward. This mechanism is consistent with the strong positive correlation between mongolism and advancing maternal age (*3*, *4*).

The risk of leukemia in mongolism is increased at all ages and is at least 20 times greater than the rate for the general population (*5*). The cell-type distribution of leukemia in mongols is reported to be similar to that of other leukemic children (*6*), but further cytologic study with uniform diagnostic criteria is needed. The genetic defect in mongolism causes other hematopoietic disturbances, such as defective lobing of polymorphonuclear leukocytes (*6*) and occasionally a transient myeloproliferative disorder at birth resembling acute leukemia (*7*). The affected infants often survive this leukemoid or "leukemia-like" disorder, believed to result from an ineffective regulation of the production and maturation of cells in the myeloid series of the bone marrow (*7*). Another abnormality of chromosome #21, with shortening of the long arm (the Ph^1 chromosome), is found in most patients with chronic myelogenous leukemia (*8*). Aberrations of this chromosome, therefore, are seen both in hyperplastic and neoplastic myeloproliferative disorders.

Bloom's Syndrome

This rare disorder is manifested by low birth weight, dwarfism, unusual facies, and a photosensitive telangiectatic erythema on the face. The chromosomes of cultured blood cells tend to break and rearrange (*9*). The occurrence of sibship aggregation, unaffected parents, and a high frequency of consanguinity points to autosomal recessive inheritance. There is no explanation for the 3:1 male excess, but ethnic predilection (a high proportion of families with Jewish ancestry) is consistent with a recessive pattern. Among 23 patients discovered with Bloom's syndrome by 1966, 3 developed acute leukemia (specified as myelogenous in 2) at ages 13, 23, and 25 years, respectively (*9*). Although the frequency of leukemia appears quite high in Bloom's syndrome, the true risk would be clarified by recognition of additional affected children and by a longer follow-up. (This generalization applies also to each condition discussed below.)

Fanconi's Syndrome

This disorder combines constitutional aplastic anemia with a variety of congenital anomalies, such as growth retardation, skeletal defects of the forearms, hyperpigmentation, genitourinary-tract defects, and deformed ears. As in Bloom's syndrome, cultured cells show chromosomal breaks and rearrangement, and there is an autosomal recessive mode of inheritance (*10, 11*). The cytogenetic abnormalities appear to precede the pancytopenia, which usually develops in children between 5 and 9 years of age (*10*). Bloom and associates reported 2 patients with Fanconi's syndrome in association with acute monocytic leukemia, ascertained during a study of 14 families with constitutional aplastic anemia (*10*). A relatively high frequency of leukemia has also been reported among relatives of patients with Fanconi's syndrome (*12*).

Klinefelter's Syndrome

This syndrome consists typically of gynecomastia, eunuchoidism, hypoplastic testes with aspermatogenesis, elevated urinary gonadotropins, and at least one extra X chromosome. As in mongolism, the cytogenetic aberration appears to result from meiotic nondisjunction. The disorder has been described in association with leukemia in four cases (*3, 13–15*), and lymphoma in three (*16–18*). It is of interest that, in the absence of any somatic anomalies, a patient with Klinefelter's karyotype (XXY) and 3 other children with abnormal chromosomes (a probable XYY, an elongated Y, and an F-trisomy) were found in a cytogenetic survey of 25 nonmongoloid children with leukemia (*15*). Since sex-chromosome anomalies such as Klinefelter's syndrome sometimes affect patients who appear clinically unremarkable, the concurrence with leukemia may be more frequent than the scattered case reports suggest.

D-Trisomy

Among the severe congenital anomalies observed in this syndrome are developmental retardation, arhinencephalia, defects of the heart and kidney, facial clefts, and microphthalmia. The disorder is due to an extra chromosome of the 13–15 (D) group and, as in mongolism and Klinefelter's syndrome, probably has its origin in meiotic nondisjunction. Recently, several hematologic abnormalities have been detected in this syndrome, including elevated levels of fetal hemoglobin and an increased number of nuclear projections in neutrophils (*19*). Although most children with this syndrome are stillborn or die soon after birth (*20*), two infants have been reported with acute myelogenous leukemia (*21, 22*). In addition, an adult female with chronic myelogenous leukemia was found to be a

D/D translocation carrier (*23*). An estimate of the true frequency of leukemia in this syndrome is especially dependent on improved survival of a sufficient number of cases beyond infancy.

CONGENITAL IMMUNOLOGIC DEFICIENCY

Whereas the congenital chromosomal disorders appear to predispose to leukemia, a predilection to lymphomas has been described among the syndromes of immune deficiency (*2*, *24*, *25*).

Ataxia-telangiectasia

This condition features a gradually progressive cerebellar ataxia, telangiectasia of the skin and conjunctiva, and susceptibility to sinopulmonary infection. Immunologic deficiency is manifested by low serum levels of IgA immunoglobulin, impairment of delayed hypersensitivity and lymphocyte transformation, and hypoplastic thymic and lymphoid tissue (*26*, *27*). There is an autosomal recessive pattern of inheritance and a definite propensity to the development of lymphoreticular neoplasms, such as lymphosarcoma, reticulum cell sarcoma, reticuloendotheliosis, and Hodgkin's disease. Among 101 case reports reviewed by Boder and Sedgwick (*28*) in 1963, 3 patients had lymphoid malignancies. Subsequently, lymphoma was reported in 10 additional cases (including 3 siblings) (*29–34*), and acute leukemia in 3 instances (including 2 siblings with lymphatic leukemia) (*34*, *35*).

Wiskott-Aldrich Syndrome

This syndrome is characterized by the triad of eczema, thrombocytopenia, and a marked susceptibility to infection. Immunologic deficiency consists mainly of a decrease in IgM immunoglobulin levels, impaired delayed-hypersensitivity responses, and hypoplastic thymic and lymphoid tissue. Transmitted in a sex-linked recessive manner, the disorder was reported in slightly more than 60 males by 1967. The frequency of lymphoreticular neoplasms seems increased, since 6 patients (including two siblings) have been observed with such tumors (*36–39*). An additional patient was reported with myelogenous leukemia (*37*).

Agammaglobulinemia and Related Defects

Congenital sex-linked agammaglobulinemia, with impaired synthesis of one or more immunoglobulins, appears to have an elevated frequency of lymphoreticular malignancy. Among 24 children studied with congenital agammaglobulinemia, Page and associates (*40*) reported that one developed malignant lymphoma and another succumbed to acute lymphocytic leukemia. The same condition has been reported with chronic monomyelogenous leukemia (*41*).

Scattered case reports implicate other forms of immunologic deficiency. For example, a boy with hypogammaglobulinemia (type uncertain) developed reticulum cell sarcoma (*42*); a phenotypically male child with congenital hypogammaglobulinemia, XX/XY lymphoid chimerism, and thymic alymphoplasia showed evidence of a leukemic cell line (*43*); and a male infant with thymic aplasia and hypogammaglobulinemia was diagnosed with lymphoma (*44*).

Chediak-Higashi Syndrome

This uncommon inherited disorder is characterized by hypopigmentation, pancytopenia, neural abnormalities, abnormal granulations in leukocytes, and decreased resistance to infection. There is no evidence for a primary immune deficiency, as found in the above disorders; instead, the susceptibility to infection may be related in some manner to the large lysosomal granules observed in circulating leukocytes (*45*). Determined by an autosomal recessive inheritance, 36 cases were reported by 1967 (*46*). A round-cell infiltration consisting mostly of immature lymphoid cells and histiocytes has been observed frequently in the organs of patients with this disease. In 4 patients the infiltration may have "progressed" to a phase that was clearly malignant or lymphomatous (*47–49*).

ETIOLOGIC IMPLICATIONS TO LEUKEMIA AND LYMPHOMA

The constitutional disorders highlight certain characteristics which appear to have a causal role in leukemia and lymphoma and provide opportunities for further research into the origins of these neoplasms.

Chromosomal Abnormalities

Leukemia occurs excessively in aneuploid conditions, such as mongolism and possible D-trisomy and Klinefelter's syndrome. These anomalies and childhood leukemia increase in frequency with advancing maternal age, suggesting that the disorders are related by a common mechanism of meiotic nondisjunction (*3, 4, 50*). Moreover, a variety of congenital defects with aneuploidy appear to cluster in some families (*50*), and occasionally include childhood leukemia as part of the aggregation (*51*). That aneuploidy and leukemia represent a cause-and-effect sequence is suggested by the observation (*15*) that 3 of 25 leukemic children without congenital defects had extra chromosomes by prezygotic origin (F-trisomy, XXY, and probable XYY.) The finding of excessive chromosomal breakage among relatives of an infant with D-trisomy and leukemia (*22*) provides further evidence of a relation between chromosomal abnormalities and leukemogenesis.

The notion that chromosomal aneuploidy or breakage accounts for the susceptibility of leukemia in general is strengthened by the observation of similar cytogenetic defects in leukemic patients (*52*) and among individuals having an "acquired" predisposition to leukemia. Chromosomal abnormalities, often long-lasting, have been documented after exposure to known or suspected environmental leukemogens: radiation (*53*), benzene (*54*), and recently chloramphenicol (*55–58*). Similar aberrations have been observed in hematopoietic disorders suspected of carrying an excessive risk of leukemia, such as certain forms of refractory anemia (*59, 60*), polycythemia vera (*61*), and possibly pernicious anemia (*62–64*). *Chronic* myelogenous leukemia may be another disease which—on the basis of chromosomal defect (rather than progression of the proliferative disorder)—is at high risk of *acute* leukemia (*65*). These observations taken together support the concept that chromosomal abnormalities are an integral part of the leukemogenic event and not purely coincidental.

Immunologic Deficiency

The repeated concurrence of congenital immune disorder and malignant lymphoma is often cited as evidence that the immunologic deficiency contributes in some manner to the origins of lymphoma. Altered or defective immunity is prevalent at all stages of the lymphomatous process and characterizes the various autoimmune or rheumatic disorders which seem to occur excessively with lymphoma later in life (*24, 25, 66–68*). Immunologic deficiency, autoimmune disease, and lymphoma tend to aggregate in certain families as well as individuals (*24, 66*), and immunologic deficiency has been postulated to be the basis for the autoimmune and neoplastic disorders (*67, 68*). Recently, animal models have been developed to investigate the lymphoproliferative disorders as they evolve from states of immunologic abnormality. For example, these neoplasms have complicated 1) the graft-versus-host reaction in mice (*69*) and the unusual autoimmune disease in the NZB strain (*70*); 2) the virus-induced Aleutian disease of mink (*71*), as manifested by extreme plasmocytosis and hyperglobulinemia; and 3) a related dysproteinemia in ferrets (*72*). Thus, the constitutional disorders are consistent with other evidence implicating impaired immune mechanisms in the origins of lymphoma.

Combined Defects

Although evidence of a consistent chromosomal or immunologic defect was used to distinguish two groups of congenital disorders, these defects seem to overlap at times. For example, immunoglobulin deficiency has been observed with the chromosomal aberration in Bloom's syndrome (*73*) and chromosomal breakage with the immune deficiency in ataxia-telangiectasia (*35*). These observations are especially relevant to Fialkow's recent hypothesis (*74*) that immunologic reactions induce neoplasia through chromosomal changes.

Genetic Factors

The constitutional disorders, being genetic in origin, implicate a genetic influence in leukemia and lymphoma. "Leukemogenic genes" may explain the occasionally striking familial aggregation of leukemia that occurs with (*35*) or without (*75, 76*) a constitutional disorder. The influence of inheritance may be measured by the observation that identical twins of children with leukemia have a concordance rate of about 20–25%, with no concordance apparent for fraternal twins (*77*). The slight increase in leukemia among siblings of affected children is consistent with a genetic influence, or possibly a common environmental exposure (*3*). Genetic factors may also account for the reported relation of leukemia to certain serum haptoglobin types (*78*) and perhaps to other gene-controlled characteristics. Further search should be made for constitutional "leukemogenic" disorders in addition to those associated with a visible cytogenetic or immunologic defect. An example may be osteogenesis imperfecta, since a pair of siblings has been described with the combination of this syndrome and acute lymphocytic leukemia of childhood (*79*).

Further Applications

Animal counterparts.—The pathogenic relation between constitutional diseases and neoplasia would undoubtedly be clarified by the comparative study of other spontaneously affected species. The Chediak-Higashi syndrome occurs in mink and cattle (*80*), and Klinefelter's syndrome appears to have a feline counterpart (*81*). There may be natural animal models to other constitutional diseases which, if recognized, would provide great impetus to further research on these diseases.

Experimental system.—Tissues from patients with constitutional disorders provide an intriguing system for the experimental investigation of oncogenic mechanisms. In preliminary studies, skin fibroblasts from individuals with Fanconi's syndrome (*82*) and mongolism (*83*) showed a greater tendency to *in vitro* transformation by the oncogenic virus, SV40, than did normal cell cultures. Extension to other "high-risk" diseases will suggest the extent to which this technique can predict *in vivo* susceptibility to cancer.

Field studies.—Future attempts at identifying etiologic agents in human leukemia and lymphoma may involve the longitudinal study of individuals over a period of time that covers the critical phase of tumor induction. Such a project would be extremely difficult to carry out, perhaps impossible, if all persons had an identical risk of developing these otherwise uncommon cancers. Epidemiologic and experimental advantages would result from the use of patients at exceptionally high risk of leukemia and lymphoma, such as those affected with certain constitutional diseases.

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Non-Neoplastic Lesions of Lymph Nodes of Man to be Differentiated From Lymphomas[1]

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SUMMARY

The differentiation of reactive processes from lymphomas in lymph nodes requires correct removal of a representative lymph node and its proper fixation, staining, and sectioning to provide technically ideal microscopic sections. The correct interpretation of these sections depends on an appreciation of the range of histologic changes that may be produced by reactive processes, definite criteria for the malignant lymphoid diseases, and a systematic approach to the study of lymph nodes, including differential diagnosis. It is essential to remember that every lymphoma may be simulated by a reactive process and that the diagnosis of lymphoma is based on morphologic features rather than clinical findings.—Nat Cancer Inst Monogr 32: 233–255, 1969.

IN CONTRAST to the large body of material on the neoplastic processes of the lymph nodes, surprisingly little has been written about the far more common non-neoplastic lesions of these structures. Most published papers concerned single entities or presented relatively brief comments regarding the differential diagnostic features of non-neoplastic diseases in connection with discussions of malignant neoplasms (*1*). Only two investigators (*2, 3*) have written in any depth on the subject as a whole.

This discrepancy between the relative incidence of malignant and non-neoplastic (reactive) lesions of lymph nodes and the amount written about each seems best explained by the reason for removing a lymph node in the first place, *i.e.*, to determine the presence of a process that is amenable to treatment. Of the non-neoplastic or reactive processes discussed herein, only one, syphilis, requires treatment of the patient. Further, although everyone is satisfied if the process is a reactive one of known etiology, a lingering suspicion that the node reviewed was not representative remains if, as is much more often true, the etiology of the reactive process is unknown. This attitude is supported by Saltzstein's (*4*) study of 68 patients from whom lymph nodes were removed because of local or generalized enlargement. Of the 68 patients, 35 had nondiagnostic lymph node biopsies, 6 of whom subsequently were proved to have lymphoma.

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The correct diagnosis of nodal disorders depends on careful control of all phases of the study, from the selection of the lymph node to be removed until the interpretation of the microscopic slides prepared from the lymph node is completed. For this reason, the present discussion is concerned with technical factors and the general approach to interpretation of lymph nodes, in addition to specific reactive processes and the differential diagnostic features. Included are only those reactive processes most frequently confused with malignant lymphoma or Hodgkin's disease.

NORMAL ANATOMY OF A LYMPH NODE

An understanding of the histologic changes in lymph nodes requires an understanding of their normal anatomy. Lymph nodes are enveloped in a capsule, which is interrupted only at the hilum by the blood vessels entering and leaving. For our purposes, the internal anatomy of the lymph node may be divided into three parts: 1) The *sinuses*, which lie beneath the capsule and ramify through the substance; 2) the *reactive follicles*, which are usually most prominent just inside the subcapsular sinus; and 3) the *pulp*, or remaining lymphoid tissue within the capsule. One must remember that the sinusoids are present in the medulla of the lymph node, since focal proliferations of the sinusoidal histiocytes, particularly in this region of the lymph node, are more likely to simulate lymphoma. When a question arises as to whether a focus of lymphoid tissue represents a lymph node or a collection of inflammatory cells, the presence of a capsule with a subcapsular sinus is the most reliable indication that the tissue in question is a lymph node.

TECHNICAL DIFFICULTIES IN INTERPRETATION

It is a constant source of amazement to those with a special interest in lymph node interpretation that the influence of purely technical factors on the diagnosis of disease conditions in the nodes is so little understood. This has been particularly true since Robb-Smith (*2*) discussed many of these factors in 1947, and Gall and Rappaport (*5*) discussed them in more detail in 1958. Errors in technique are unquestionably the single most important cause of diagnostic difficulties in diseases of the lymph nodes. From my experience with lymph node problems, I suspect that the major difficulty in establishing a diagnosis of lymphoma in the six cases of Saltzstein's (*4*) series could be attributed to technical errors. Although the first three of the factors enumerated below are not directly under the pathologist's control, it is his duty to see that they are known to the surgeon, internist, and radiotherapist, in order that the difficulties to which they can lead may be reduced to a minimum. The factors to be considered are:

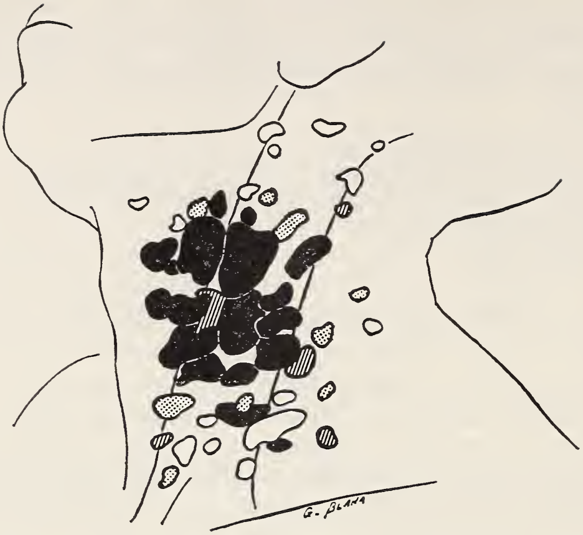
1. Poor selection of the biopsy site.
2. Poor selection of the lymph node or nodes at the chosen site.
3. Improper removal of the lymph node or nodes by the surgeon.
4. Poor fixation of the specimen.
5. Improper cutting.
6. Improper staining.

The selection of a biopsy site is obviously no problem if only one node or one group of nodes is enlarged. If more than one site may be chosen, either a lower cervical or axillary lymph node is best. Reactive processes in lymph nodes in the parotid gland, the submandibular area, and the inguinal areas are commonly exuberant, and may be atypical because of inflammatory processes of the gingiva and surrounding areas drained by the parotid and submandibular groups, and of the legs and genitalia drained by the inguinal group.

To be as certain as possible that the node removed is representative, the largest possible lymph node should be excised. It has been my experience that surgeons, if not cautioned, will usually remove the most accessible lymph node; unfortunately, this may be too small to represent the disease process. Slaughter *et al.* (*6*) have shown that, if a radical lymph node dissection were performed in Hodgkin's disease and the histologic changes in each node were mapped (text-fig. 1), one or more normal nodes or ones exhibiting only a slight degree of atypia could be found adjacent to those having diagnostic histologic features. The only gross indication that a node was representative was its size, the largest node being most likely to be representative. We, also, have found this to be true in reactive processes of known and unknown etiology, and in all types of lymphoma.

In addition to the choice of the largest lymph node, it is also important that the node be removed in one piece with the capsule intact. If the node is removed in fragments, one loses an appreciation of the relationship of the normal anatomic structures to each other and to any process that may be present. Disturbing areas in benign hyperplastic lymph nodes are much more easily evaluated in the intact node than if they appear in one or more pieces of a fragmented lymph node; what should be correctly identified as an exuberant and somewhat disturbing reactive process may then be misinterpreted as a malignant process. Similarly, if a distinction between a poorly differentiated metastatic carcinoma and histiocytic reticulum cell sarcoma is difficult, the availability of an intact node simplifies the problem; in a lymph node not completely filled with carcinoma, the sinusoids will be prominently involved.

Whereas the technical factors mentioned thus far are beyond the pathologist's direct control, those remaining are under his direct control.



TEXT-FIGURE 1.—Detail of nodal involvement in specimen from neck dissection for Hodgkin's disease. *Black nodes* represent a full-blown Hodgkin's pattern. The histologic pattern of the *white nodes* was normal, and in the *cross-hatched* nodes the only deviation from normal was an increase of eosinophils. The *striated nodes* exhibit the pattern described as "early Hodgkin's," or "paragranuloma."

From Slaughter, D. P., Economou, S. G., and Southwick, H. W.: Ann Surg 148: 705–710, 1958, with permission of Dr. Slaughter and *Annals of Surgery.*

The most important of these technical factors is proper fixation. If the sections are too thick, others may be cut, and improperly stained sections can be restained. Nothing, however, can be done with an improperly fixed specimen. Upon receipt of the specimen, imprints should be prepared by cutting off one end of the lymph node, if only one is removed, or one end of the largest, if several nodes are obtained. For the most accurate imprints, this should be done as rapidly as possible. Immediately thereafter, the node should be placed into the fixative. The choice of fixative is less important than is familiarity with the fixative used. It is discouraging to attempt to evaluate lymph nodes distorted by handling during fixation in Zenker's, Carnoy's, or some other fixative that requires a special technique. Buffered formalin will give satisfactory results without the bother or dangers of special handling. The lymph node or nodes should be separated from attached adipose tissue and placed in formalin with the capsule intact, aside from the end removed to obtain imprints. After remaining in formalin for an hour or so, the node should be cut into parallel sections about 3 mm thick to insure proper penetration of the fixative. The nodes should be sufficiently small enough to permit their placement, with the capsule attached, on a glass slide. If nodes are cut before being placed in fixative, the pulp tends to bulge from the capsule and produce distortions. The sections should be replaced in the fixative and allowed to remain overnight, even though this means the diagnosis will be delayed an additional day; the quality of the sections will amply justify the delay. If a diagnosis the following day is urgent, a portion of the largest node can be processed overnight.

In sections cut too thick, the cells overlie one another; thus, the cytologic details, so essential to lymph node interpretation, are difficult to detect. The sections should be one cell in thickness, or 6–8 μ. Therefore the technician should take special care in cutting. The thickness of a section can be determined only under the microscope. The setting on the microtome does not necessarily reflect the true thickness.

Proper staining is also essential for identification of the cytologic details. A light stain is required for delineation of the nuclear features. The problem is usually one of overstaining.

BASIC PRINCIPLES OF THE STUDY OF LYMPH NODES

In the histologic study of lymph nodes, several basic principles should be observed. Failure to appreciate these principles explains many of the diagnostic difficulties encountered. It is assumed that the lymph node under examination is representative of the disease process.

The first principle is *the necessity of having technically excellent sections,* as already discussed. If the sections are not of highest quality, one has nothing to which to apply the other principles.

Second, *the frozen section technique and needle biopsies should be used to evaluate lymph nodes only* if the presence of lymphoma is unlikely. Frozen sections cannot be cut sufficiently thin for proper evaluation, even with the use of the cryostat. Further, because of the changes in the tissue produced by freezing, it is impossible to obtain a satisfactory section subsequently by the usual paraffin-embedding technique.

Needle biopsy is inadequate as a means of providing tissue; the specimen is usually too small to be representative, and the pathologist is unable to relate the changes to the normal anatomic landmarks. In addition, the biopsy procedure itself distorts the tissue.

Third, *a normal lymph node is a reacting one; the pattern of reaction depends on the stimulus and its reacting counterpart.* The problem here, of course, is that we do not know the range of reaction, primarily because too few studies have been made of lymph nodes in known disease states. The pattern of reaction varies not only with the stimulus and its means of reaching the lymph node, by lymphatics or bloodstream, but also with the reacting capabilities of the lymph node itself. Young persons, particularly those in the first two decades of life, tend to exhibit reactive follicular hyperplasia as a response to many stimuli, whereas persons over 60 years old tend to have a predominance of lymphocytes in the pulp, the follicular component being much less prominent. If the hematopoietic system cannot respond normally because of nutritional deficiency or disease, or has been damaged by a previous illness, such as measles, or by drugs, irradiation, or some other cytotoxic agent, the reaction to a given stimulus will differ from that expected. Thus, atypical cytologic changes in a lymph node may represent the response of an inadequate or damaged hematopoietic system, rather than a primary neoplastic process.

Closely related is the fact that *reactive processes may obscure the nodal architecture.* Overreliance on change in nodal architecture as a criterion of malignancy accounts for many erroneous diagnoses of malignant processes in lymph nodes. Study of a large number of radical node dissections for malignant neoplasms of various types, whether or not radiotherapy has been administered previously, clearly reveals that hyperplastic processes may obliterate the internal architectural features of the nodes and involve the capsule and adjacent tissues (fig. 1). Interestingly, overreliance on architecture may be a two-edged sword, since in early involvement of lymph nodes by granulocytic or undifferentiated (stem cell) leukemia and some forms of Hodgkin's disease, the nodal structure may be only slightly altered.

From study of lymph nodes exhibiting a variety of known reactive processes, it becomes apparent that *one cannot apply the criteria for malignant disease in other tissues to the study of lymph nodes.* Whereas the presence of mitoses, invasion, or abnormal individual cells generally indicates a malignant process in tumors of the soft tissues or epithelial neoplasms, all these features may be present in benign conditions of lymph nodes (fig. 2). This points up the need for positive criteria in dealing with all proliferative processes of the hematopoietic system.

Basic to a correct interpretation of changes in the lymph nodes is awareness of the fact that *every pattern of lymphoma may be simulated by a reactive process.* This may be attributable to cytologic atypia already mentioned, or to the production of histologic patterns similar to those of lymphoma, as will be illustrated.

A malignant lymphoma is essentially a proliferation of a single cell type and is a cytologic entity; the cells may have a nodular or diffuse growth pattern. The cells may vary somewhat in size and shape, but multiple cell types are not present.

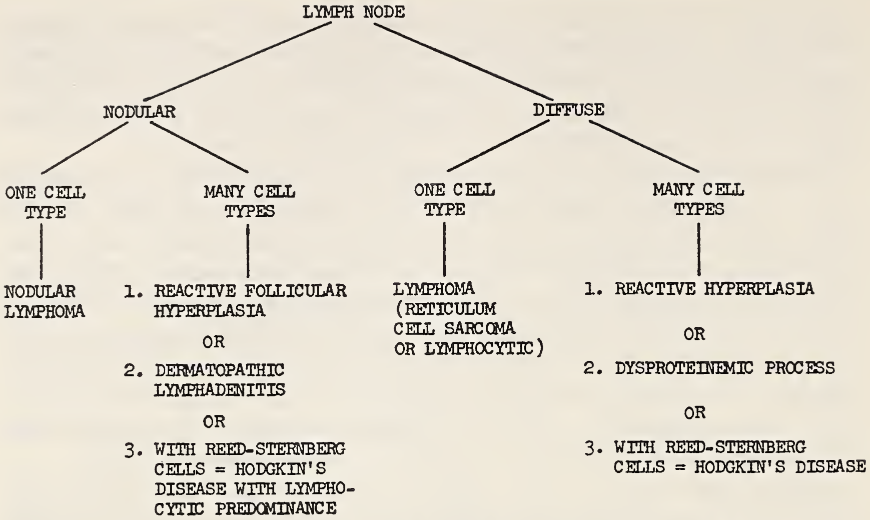
In Hodgkin's disease, the lymph nodes contain a polymorphic cellular infiltrate. Reed-Sternberg cells with large nucleoli are essential to the diagnosis. Since one may find mononuclear cells with relatively large nucleoli in reactive processes, the distinction will depend on the presence of diagnostic Reed-Sternberg cells (fig. 3). Fortunately, reactive processes do not produce polyploid and large nucleoli. As previously reported (7), the variation in the cellular components in Hodgkin's disease is a reflection of the prognosis.

Although enlargement of lymph nodes is one of the usual reasons patients give for presenting themselves to physicians, *the size of a lymph node is no criterion of malignant* disease. One of the largest nodes ever reported (*8*) was a reactive lymph node 17 × 12 × 8 cm, which had been present for 25 years and was removed for cosmetic reasons.

Obviously, *the diagnosis of lymphoma and Hodgkin's disease should be based on morphologic, rather than clinical, findings—on positive criteria, rather than on the process of exclusion.* Lymph nodes should be examined objectively, the pathologist having no clinical information that might influence his interpretation. If the pathologist has preconceived ideas of what the diagnosis should be, he could easily read this diagnosis into the changes present. He should force himself to write down his objective opinion, then study the clinical information, and examine the sections again as a precaution against overlooking anything. He always looks closest at sections if he has no knowledge of the patient's history.

Finally, lymph nodes must be studied systematically. This involves the study of the entire section at scanning (30–40×) and low (100×) magnifications and of selected areas under high (430×) magnification. It also involves an application of the principles previously mentioned, with consideration of the differential diagnostic features (text-fig. 2). In this scheme, one first determines that the biopsy specimen is a lymph node by the presence of a capsule and a subcapsular sinus. The sections are next examined under low power to determine whether the process is diffuse or nodular, and then whether this process is composed of one cellular type or of multiple types. If the cells are of multiple types, the presence or absence of Reed-Sternberg cells is noted. In this somewhat simplified scheme, no provision is made for metastatic disease. This should be mentioned, because the differentiation between metastatic tumor, reticulum cell sarcoma, and a reactive process occasionally may be a problem. Since metastatic tumors usually prominently involve sinusoids, and reticulum cell sarcomas rarely involve sinusoids, this distinction is usually possible. Reactive processes, however, may also produce this pattern, as a result of a proliferation of sinusoidal histiocytes in a drainage reaction, *i.e.*, a reaction to an infection in which the organisms or their products drain to the regional lymph nodes through the lymphatics and stimulate proliferation of the sinusoidal lining cells. This differentiation requires proper identification of the cell type involved.

Before specific reactive processes that may simulate lymphoma or Hodgkin's disease are discussed, the general characteristics of reactive hyperplasia of lymph nodes must be considered. This general term refers to proliferative processes arising as a reaction to different stimuli, such as an infectious agent or its product, a chemical or a hormone. In a few cases, the initiating agent or group of agents can be determined from the histologic changes present.


LYMPH NODE
NODULAR
DIFFUSE
ONE CELL TYPE
MANY CELL TYPES
ONE CELL TYPE
MANY CELL TYPES
NODULAR LYMPHOMA
1. REACTIVE FOLLICULAR HYPERPLASIA
OR
2. DERMATOPATHIC LYMPHADENITIS
OR
3. WITH REED-STERNBERG CELLS = HODGKIN'S DISEASE WITH LYMPHOCYTIC PREDOMINANCE
LYMPHOMA (RETICULUM CELL SARCOMA OR LYMPHOCYTIC)
1. REACTIVE HYPERPLASIA
OR
2. DYSPROTEINEMIC PROCESS
OR
3. WITH REED-STERNBERG CELLS = HODGKIN'S DISEASE

Text-figure 2.—Schematic approach to the diagnosis of disease of lymph nodes.

The variety of reactive processes is limited by the number of anatomic structures or cellular types in a lymph node. The characteristics of a fully developed reactive process are listed in table 1. Except for those noted, all elements of the lymph node are generally involved in the reaction. Different stimuli or types of stimuli, however, usually produce a more prominent proliferation in one or more of the three parts of the lymph node, or of one cell, or of both; in its fully developed stage, this combination of histologic changes becomes characteristic of the process in question. Unfortunately, too many of the reactive processes are nonspecific. The confusion of reactive with neoplastic processes can be attributed, at least partially, to the pathologist's failure to remember that the reaction is of a biologic, rather than of a chemical nature. Consequently, the reactions vary in different persons, depending on many variables, such as the age of the patient, reactive capacity of the lymphoid tissue, magnitude of the stimulus, stage of the process when the lymph node was removed, and the previous experience of the patient with the stimulus in question. When the response of one anatomic structure or one cell type is significantly more pronounced than usual, the likelihood of confusion with a malignant lesion increases.

Reactive processes of lymph nodes which simulate lymphoma may be classified according to their etiology (table 2) or according to the type of lymphoma simulated (table 3). The first method is especially useful, in that it indicates the conditions of known etiology that can simulate lymphoma. Although recognition that a process is reactive is more important, recognition of a specific etiology virtually eliminates the possibility that the lymph node obtained was not representative of the disease.

TABLE 1.—Characteristics of reactive hyperplasia of Lymph Nodes

A. *Proliferation of sinusoidal histiocytes*
B. *Proliferation of reactive follicles*
C. *Proliferation of pulp elements*
   1. Mixed cellular proliferation
      a. Histiocytes
      b. Lymphocytes
      c. Undifferentiated reticular cells
      d. Plasma cells
      e. Eosinophils (at times)
      f. Neutrophils (at times)
   2. Endothelial cells of small blood vessels increasing in size and number

The fact that the list of reactive processes of unknown etiology is shorter is deceptive, since more than 95% of reactive lymph nodes are in this group. The list is short because each of the descriptive terms used for the first three represents prominence of one of the three anatomic structures in a lymph node, *i.e.*, the follicle, sinusoids, and pulp, respectively. The fourth entity appears to represent a late stage of reactive follicular hyperplasia. These are broad categories from which we must attempt to separate those processes that can be proved to be of a known etiology. Only the last extremely rare entity, allergic granulomatosis, is not descriptive of the anatomic subdivision that is most prominent in the reactive process.

Classification of the reactive processes according to the malignant lesion simulated emphasizes, once again, the need for a differential diagnostic approach to lymph node interpretation. This approach is, of course, the basis for the schematic diagram in text-figure 2. Neither this list nor the schematic approach to lymph node diagnosis includes metastatic carcinomas, which must be considered in the differential diagnosis, particularly of reticulum cell sarcoma.

TABLE 2.—Reactive processes of lymph nodes which simulate lymphoma, etiologic classification

A. *Known etiology*
   1. Rheumatoid arthritis
   2. Secondary syphilis
   3. Toxoplasmosis
   4. Infectious mononucleosis
   5. Dermatopathic lymphadenitis
   6. Postvaccination lymphadenitis
   7. Herpes zoster (viral)
   8. Dilantin lymph node hyperplasia
B. *Nonspecific*
   1. Reactive follicular hyperplasia
   2. Reactive hyperplasia with prominence of sinusoidal histiocytes
   3. Chronic nonspecific lymphadenitis
   4. Angiofollicular (mediastinal) lymph node hyperplasia
   5. Allergic granulomatosis

TABLE 3.—Reactive processes of lymph nodes which simulate lymphoma, classified according to the malignant process simulated

A.? *Nodular lymphoma*
    1. Rheumatoid arthritis
    2. Syphilis
    3. Reactive follicular hyperplasia
    4. Angiofollicular (mediastinal) lymph node hyperplasia
    5. Dermatopathic lymphadenitis
B.? *Diffuse lymphoma or Hodgkin's disease*
    1.? Lymphocytic lymphoma
        a. Chronic nonspecific lymphadenitis
    2.? Reticulum cell sarcoma or Hodgkin's disease
        a. Infectious mononucleosis
        b. Postvaccination lymphadenitis
        c. Herpes zoster (viral) lymphadenitis
        d. Dilantin lymph node hyperplasia
        e. Reactive hyperplasia with prominence of sinusoidal histiocytes
    3.? Hodgkin's disease
        a. Toxoplasmosis
        b. Allergic granulomatosis

The reactive processes that simulate nodular lymphoma are generally easier to recognize; one need only be familiar with the spectrum of changes in reactive follicles to understand the nature of the first four of the five nodular processes. In contrast, one must pay close attention to individual cells to recognize the proliferations that simulate diffuse lymphoma or Hodgkin's disease.

The non-neoplastic reaction of lymph nodes most often confused with nodular lymphoma is *reactive follicular hyperplasia.* Although associated histologic findings may lead one to suspect a specific etiology, in most cases the cause of the hyperplasia is not apparent. We do know that the lymph nodes of young persons, especially of children, exhibit this reaction to a wide variety of stimuli. As a consequence, one must be careful in making a diagnosis of nodular lymphoma in a young person; I have never seen the disease in anyone less than 20 years old, and seldom in one less than 30.

Histologically, in their proliferative phase, reactive follicles are characterized by a variation in size and shape (fig. 4) and are sharply demarcated from the surrounding lymphoid tissue. In the initial phases of the reaction and in the less severe cases, reactive follicles tend to be more prominent in the peripheral portion of the node. Unfortunately, this is generally true only in lymph nodes that are not particularly worrisome anyway. Cytologically, the reactive follicles are composed of undifferentiated reticular cells, histiocytes, and large lymphocytes having little or no nuclear atypia (fig. 5). The contrast between these cells and the small lymphocytes of the surrounding pulp accounts for the sharp demarcatio of the follicles, apparent even under low magnification. The histiocytes in the reaction centers generally exhibit active phagocytosis, the nuclear debris in the cytoplasm being responsible for the "starry sky" appearance. One typical finding in reactive follicles is the increased number of normal mitotic figures. In contrast, nodular lymphoma is characterized by round to oval nodules which have little variation in shape and, at most, only a moderate variation in size and which are evenly distributed throughout the lymph node. Cytologically, the nodules are composed of abnormal cells that may vary in size, with nuclear atypia. Both phagocytosis and mitotic figures are uncommon. The cells within the nodules may be identical to, or different from, those of the surrounding pulp, although the nodules are never as well delineated as reactive follicles. All these features are among those first listed by Custer (*9*) and later modified by Rappaport *et al.* (*10*). They are often helpful in pointing to the correct diagnosis; yet only by determining whether or not one is dealing with nodules of lymphoma cells can the distinction be made with certainty. This can be extremely difficult.

The next two entities that may resemble nodular lymphoma are systemic processes in which lymphadenopathy is widespread and frequently generalized. The changes in the lymph nodes in primary and secondary *syphilis* are similar, although the changes in the secondary stage are more likely to be confused clinically with those of lymphoma because of the presence of generalized lymphadenopathy. Also, generalized enlargement of the lymph nodes is present in 50–75% of patients with *rheumatoid arthritis* and may precede the appearance of the arthritis (*11*). In both syphilis (*12*) and rheumatoid arthritis (*11*) numerous reactive follicles are found in all parts of the lymph node (fig. 6). In rheumatoid arthritis, a marked proliferation of plasma cells in the intervening stroma is apparent (fig. 7). Since these changes may be observed in lymph nodes draining a site of chronic inflammation, they are not diagnostic of either disease. Both should be considered, however, particularly if the patient has generalized lymphadenopathy.

Angiofollicular (*mediastinal*) *lymph node hyperplasia* appears to represent the end stage of some reactive follicular hyperplasias in which many of the follicles undergo regressive changes, with hyaline deposition and vascular proliferation (fig. 8). As a result, some of the distorted follicles resemble *Hassall's* corpuscles; thus, these lesions in the mediastinum have been mistaken for a disorder of the thymus. Since they may be found outside the mediastinum (*13*), the term "mediastinal lymph node hyperplasia," as they were first called (*14*), is incorrect. In certain examples of this condition, one can observe a more or less orderly transition from clear-cut reactive follicles to quite distorted forms that are difficult to recognize as reactive follicles (fig. 9). Histologically, these lesions are readily distinguished from those of lymphoma; although numerous, the follicles are generally small and widely separated, and no abnormal nuclear changes are apparent in either the nodules or the intervening pulp. The chief problem, so far as malignant change is concerned, lies in the fact that these lymph nodes tend to be large and frequently are found in unusual locations. They may be considered malignant because of their size, even though, as already stated, this is not a reliable index of cancer.

Dermatopathic lymphadenitis (*15*) is the one nodular entity in which the nodularity is not a direct reflection of follicular hyperplasia. Hyperplasia of both the follicles and sinusoidal histiocytes may be observed, yet the most conspicuous change consists of poorly defined foci of histiocytic proliferation in the cortical portion of the lymph node (fig. 10). The histiocytes (fig. 11) contain fat and finely dispersed melanin; the latter, however, may at times be demonstrable only by special stains. Because of the contents of the histiocytes, this process has also been called "lipomelanotic reticulosis" (*16*). As the term "dermatopathic lymphadenitis" implies, these changes represent a reaction to chronic skin disease, usually an eczematoid or exfoliative dermatitis, or an erythrodermia. Mild changes of this type appear to be constantly present in persons with hyperpigmented skin, or they may be associated with even minor skin disorders. Consequently, if the diagnostic term is to be meaningful, it should be applied only to those cases in which lymph nodes are definitely enlarged.

The diffuse processes simulating lymphomas and Hodgkin's disease test diagnostic criteria most severely. In this group, the cytologic changes throughout the node must be carefully evaluated. Without a clear-cut definition of a diagnostic Reed-Sternberg cell, for example, one can visit disaster on the patient by interpreting an inflammatory process as Hodgkin's disease.

Chronic nonspecific lymphadenitis (*1*) in which lymphocytic proliferation predominates is particularly difficult to distinguish, in that it tends to develop in older individuals, in whom lymphocytic lymphoma is also more common. In chronic lymphadenitis of this type, the lymphocytes have a thin rim of cytoplasm, differ widely in size, and are associated with a varying number of histiocytes, plasma cells, and primitive reticular cells. A constant feature is the presence of prominent small vessels lined by enlarged endothelial cells (fig. 12). Lymphocytes with a conspicuous rim of cytoplasm are also present, although they are not significant in the process. Eosinophils may be observed, as in any chronic inflammatory reaction.

This condition must be distinguished from two variants of lymphocytic lymphoma. In one, the differentiation is relatively easy, since the lymphocytes are remarkably uniform in size. In the other, uniform lymphocytes are mixed with groups of cytoplasmic lymphocytes. In neither variant are other cell types prominent.

Another group of conditions are those that may be misinterpreted as Hodgkin's disease or histiocytic reticulum cell sarcoma. In the first four, the diagnostic difficulty is attributable to proliferation of atypical histiocytes or large cytoplasmic lymphocytes throughout the pulp. Either the process is interpreted as histiocytic cell sarcoma because of the prominence of the large cytoplasmic cells, or some of these cells are misinterpreted as Reed-Sternberg cells and the process is thus considered to be Hodgkin's disease. The first of these errors arises from the pathologist's failure to realize that in histiocytic reticulum cell sarcoma the overwhelming majority of the cells are abnormal histiocytes, whereas in the reactive processes less than half the cells are histiocytes or cytoplasmic lymphocytes. The second error reveals a lack of adequate criteria for diagnostic Reed-Sternberg cells (fig. 3). The necessity for lobated or multinucleated Reed-Sternberg cells for the diagnosis of Hodgkin's disease is established, as is the unreliability of mononuclear cells with large nucleoli (*1*, *7*). Both of these points were emphasized by a recent study of postvaccination lymphadenitis, in which large mononuclear cells with large nucleoli abound. In this study, 7 of 20 cases were incorrectly interpreted as Hodgkin's disease (*17*). In the last entity in this group, necrotizing lymphadenitis, irregular foci of sinusoidal histiocytes in all parts of the lymph nodes are the problem.

Infectious mononucleosis is the best known of the reactive processes that simulate lymphoma. The difficulty is more likely to arise in the somewhat atypical case, in which the patient presents with lymphadenopathy without systemic symptoms, such as fever, splenomegaly, or sore throat. The presence of these associated signs and symptoms would point so strongly to the correct diagnosis, or at least to an inflammatory process, that lymph node biopsy would be unlikely. Histologically, the lymph nodes in infectious mononucleosis exhibit proliferation of large cytoplasmic cells throughout the pulp (fig. 13). The cells (fig. 14) contain nucleoli and are usually considered cytoplasmic lymphocytes, although they resemble the cells observed in viral lymphadenitis, designated histiocytes (*1*). As a rule, reactive follicles are easily seen, since this disease affects young persons. The reactive follicles exhibit conspicuous proliferative activity, however, and thus may be partially obscured if the proliferative activity is pronounced. In thin, well-stained sections, the prominent mixture of normal lymphocytes and the absence of significant atypical cytologic features in the proliferating cells indicate a benign process.

Of all the conditions under discussion, *postvaccination lymphadenitis* appears most likely to be mistaken for a malignant disease. The enlarged nodes appear one or more weeks after the smallpox vaccination (*17*). Usually, they are located in the lateral supraclavicular or infraclavicular region, or the axilla on the side of the vaccination. They may be associated with local pain, although systemic symptoms are lacking. In a few cases, axillary, supraclavicular, and cervical lymph nodes on the same side may be enlarged (*17*). In 14 of 20 cases previously studied, the association between the vaccination and the appearance of enlarged lymph nodes was overlooked (*17*), undoubtedly because the patients reported no complaints relative to the vaccination site. Histologically, large cytoplasmic cells containing nucleoli, usually referred to as atypical histiocytes or activated reticulum cells (*1*), proliferate throughout the pulp (fig. 2) and become the most conspicuous type of cell (fig. 15). Because of their size they seem to stand out from the mature lymphocytes. As in the other processes in this group, reactive follicles may or may not be easily detected. In the study already referred to (*17*), reactive follicles were prominent only in lymph nodes removed 15 days after vaccination. Mitotic figures, as well as eosinophils and plasma cells, may often be observed. As in all reactive processes, numerous small vessels with prominent endothelial cells are readily apparent.

The lymph nodes draining infections with *herpes zoster* present histologic changes identical to those in lymph nodes draining vaccination sites (fig. 16). Since similar nodal changes develop in patients who do not have herpes zoster or infectious mononucleosis and who have not been vaccinated, it appears that they may also be induced by unidentified viruses.

Saltzstein and Ackerman (*18*) reported that Dilantin may cause a pronounced proliferation of atypical histiocytes. For this reason the possibility that a drug may be the activating agent must be kept in mind. The ease with which such proliferations may be mistaken for lymphoma can be appreciated by a study of the illustrations in the report of these authors. A history of ingestion of Dilantin or related drugs should not influence the diagnosis; patients with lymphoma may, of course, also take these drugs (*19*). One of the patients included in the original report of Saltzstein and Ackerman was subsequently proved to have lymphoma (*20*).

The final member of this group is a *variant of* ordinary *reactive hyperplasia* in which prominent *localized proliferations of sinusoidal histiocytes* frequently with small central areas of necrosis, are scattered throughout the lymph node (fig. 17). Follicular hyperplasia and proliferation of all cellular elements in the pulp are often associated, although the focal histiocytic proliferation seems to be responsible for the diagnosis of malignant disease. A benign lesion is indicated by the high incidence of central necrosis and the absence of significant nuclear atypia in the histiocytes, in addition to the lack of the already mentioned criteria for the diagnosis of histiocytic reticulum cell sarcoma and lack of Reed-Sternberg cells.

The two conditions constituting the last group point to the need for definite criteria for Reed-Sternberg cells. Both, and particularly allergic granulomatosis, also emphasize the fact that eosinophils may be present in lymph nodes, even in considerable numbers, in benign conditions as well as in Hodgkin's disease.

Toxoplasmosis is an example of a disease for which the histologic findings were recognized and described as an entity well before the etiology was discovered. In 1947, Robb-Smith (*2*) described the characteristic histologic changes in lymph nodes, and designated these changes as "lymphohistiocytic medullary reticulosis." In 1953, Piringer-Kuchinka *et al.* (*21*) also described these changes and designated the entity as "subacute nuchal and cervical lymphadenitis." In 1953, Stanton and Pinkerton (*22*) first reported the histologic changes characteristic of toxoplasmosis, although these findings were not connected with the histologic descriptions of the two previously mentioned investigators. In 1956, Siim (*23*) correlated the characteristic histologic changes with the clinical and serological findings in patients from whose excised lymph nodes *Toxoplasma gondii* was isolated; again, however, his findings were not related to the descriptions of Robb-Smith and Piringer-Kuchinka *et al.* Not until the report of Saxén and Saxén (*24*) in 1959 did these histologic findings become well known in this country and related to the descriptions of Robb-Smith and Piringer-Kuchinka *et al.*

Toxoplasmosis is exhibited by hyperplasia of sinusoidal histiocytes, follicles, and all elements of the pulp (fig. 18). The characteristic features are follicular hyperplasia and focal collections of reactive histiocytes in the pulp and follicles, the histiocytes having an abundant pink-staining cytoplasm and small nuclei containing tiny nucleoli. Groups of these histiocytes within follicles are diagnostic. Hodgkin's disease with a lymphocytic predominance (7) may exhibit a similar histiocytic proliferation, but follicular hyperplasia is rarely associated and the histiocytes do not extend into any follicles present. In addition, diagnostic Reed-Sternberg cells are lacking in toxoplasmosis.

In the past, acute toxoplasmosis was believed to be associated with systemic symptoms of variable severity. Recently, studies of lymph node biopsies (*24*) and serologic studies (*25*) have revealed that patients may have lymphadenopathy or significant serologic changes without being aware of symptoms. Naturally, asymptomatic lymphadenopathy would elicit the most clinical concern and lead the pathologist to make a diagnosis of a malignant disease. It is important to remember, as pointed out by Remington *et al.* (*25*), that an elevated titer on a single serologic test should not alone be accepted as evidence that nodal enlargement is attributable to toxoplasmosis. To establish the diagnosis of toxoplasmosis, one must have, in addition, one of the following: recognition of organisms in microscopic sections of a node, isolation of the organism by mouse inoculation, the characteristic lymph node histology, described above, or a marked rise in one of the serologic titers against the organism.

The final entity, *allergic granulomatosis* (*26*), being extremely rare, is included for reasons previously mentioned. Histologically it is manifested by a proliferation of histiocytes, most of which appear innocuous, associated with a diffuse infiltration of eosinophils and tiny stellate areas of necrosis (fig. 19). Because of the presence of histiocytes and eosinophils and the grave clinical condition of the patient, it may easily be considered as atypical Hodgkin's disease (*27*), despite the absence of cells even resembling the Reed-Sternberg type.

HEMATOPOIETIC NEOPLASMS

CONCLUSIONS

In discussing the lymph node biopsy, Robb-Smith (*2*) stated that the essentials for histologic interpretation were good visual memory, a thorough knowledge of the normal histology of the tissue to be examined, the experience gained by examination of a large number of specimens, a recognition of any disturbance of the structural pattern of the tissues examined, and a determination of the cytologic changes associated with this disturbance of structure. To these essentials should be added the need to be certain that the lymph node is representative and correctly removed, that the sections are technically ideal, and that a differential diagnostic approach is taken with consideration of all entities that could produce histologic changes similar to those present. It is assumed that the the experienced histologist would appreciate the range of morphologic changes that may be produced by reactive processes and the definite histologic and cytologic criteria for the malignant processes in lymphoid tissue. Too frequently unusual and severe reactions without any evidence to suggest an etiology are erroneously interpreted as malignant lymphoma.

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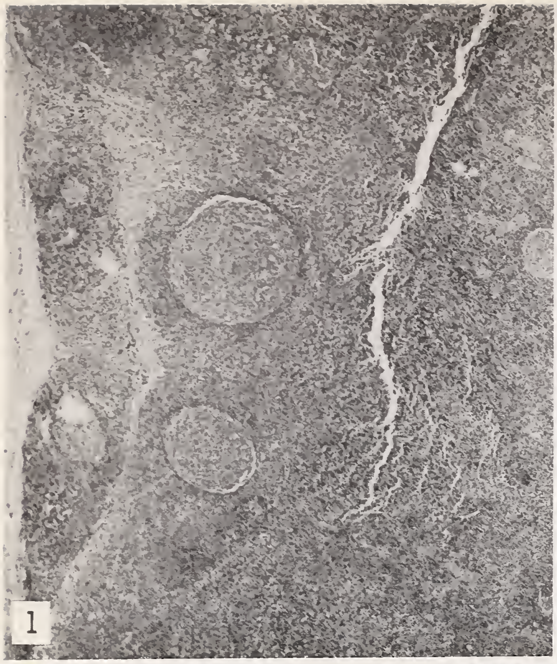
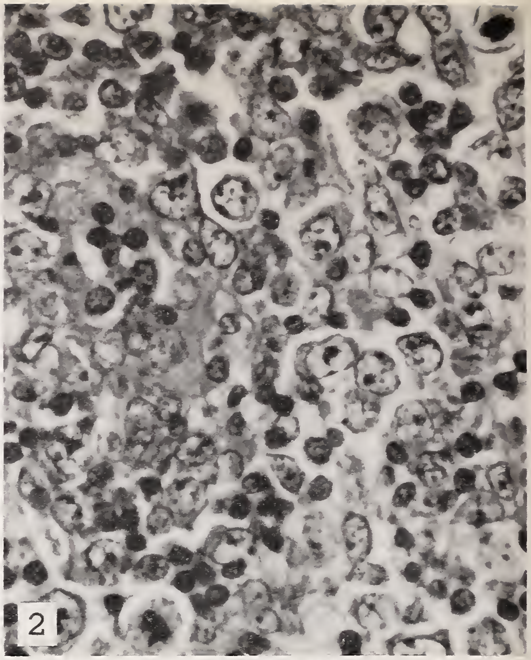
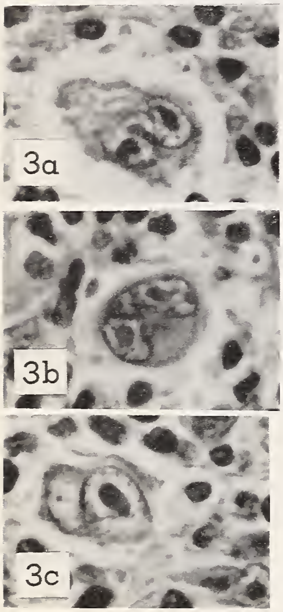
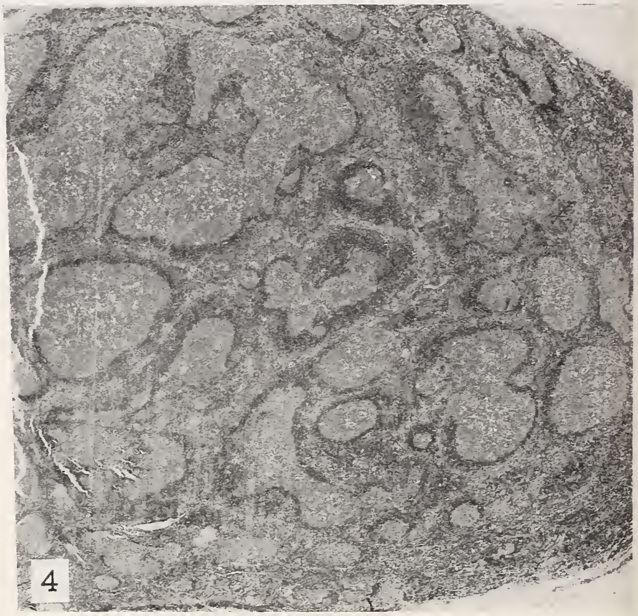








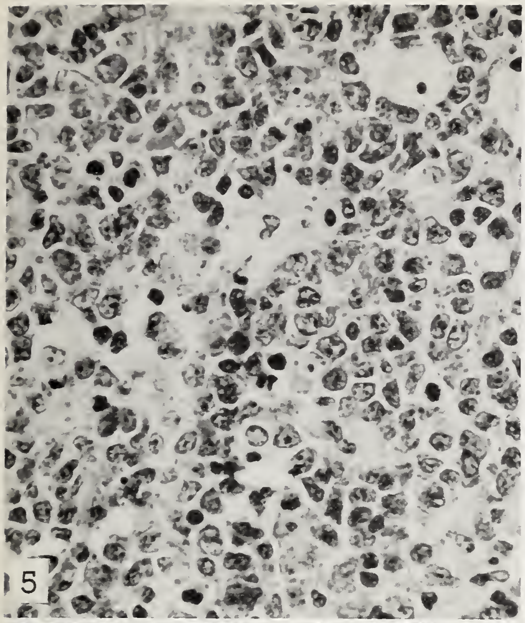
Photomicrographs prepared with the assistance of F. R. Smith, Medical Communications Department. All preparations stained with hematoxylin and eosin.

Figure 1.—Lymph node from a nodal dissection for carcinoma. Capsule at *left* contains lymphoid tissue with reactive follicles. × 9

Figure 2.—Mitotic figures and activated reticulum cells in lymph node exhibiting postvaccination lymphadenitis. × 400

Figures 3A, B, and C.—Variants of Reed-Sternberg cells. *In C,* one lobe of the nucleus partially overlies the other. × 400

Figure 4.—Reactive follicular hyperplasia. Follicles vary in size and shape and are sharply delineated from surrounding lymphoid tissues. × 9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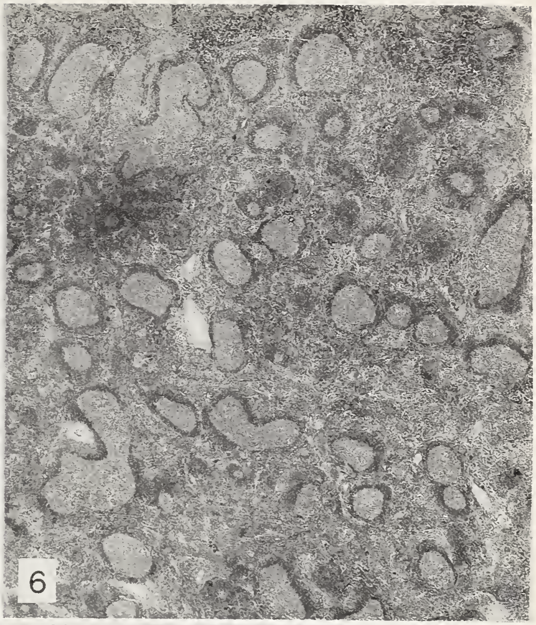
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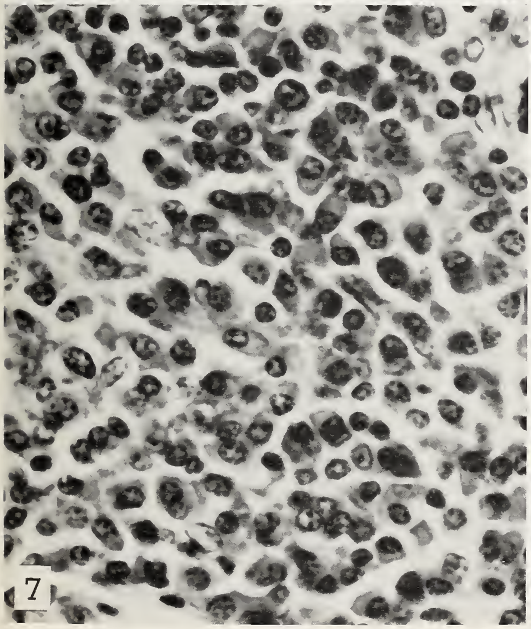
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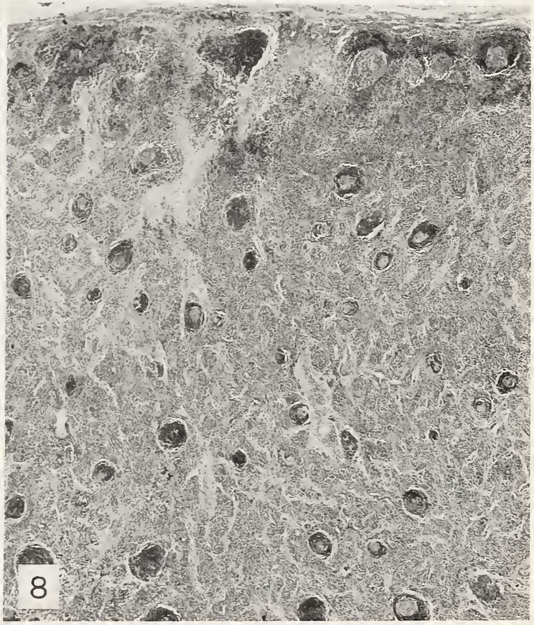
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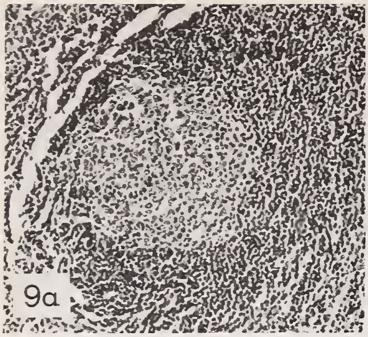
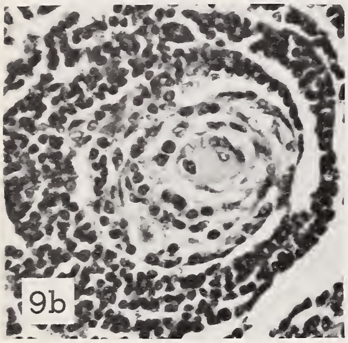
Figure 5.—Higher magnification of one reactive follicle, showing different cellular types, including active phagocytes, and numerous mitotic figures. × 300

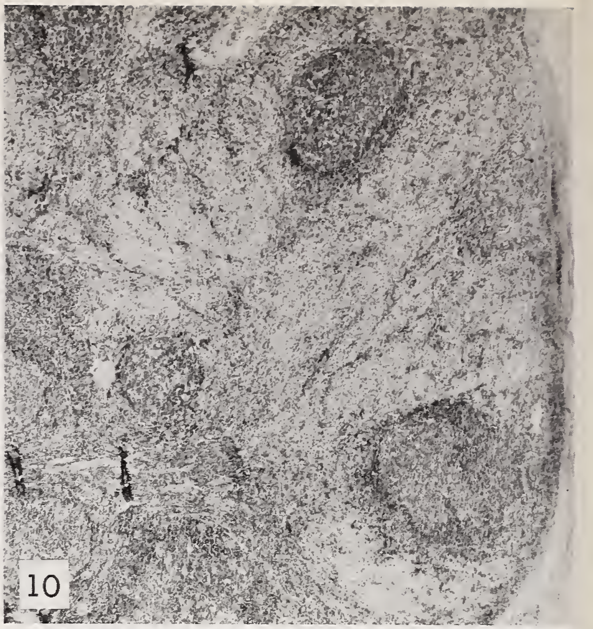
Figure 6.—Reactive follicular hyperplasia in rheumatoid arthritis. × 9

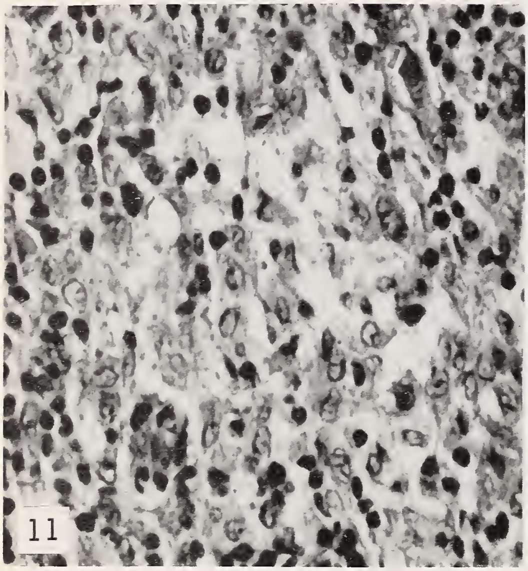
Figure 7.—Collection of plasma cells in pulp of lymph node in rheumatoid arthritis. × 400

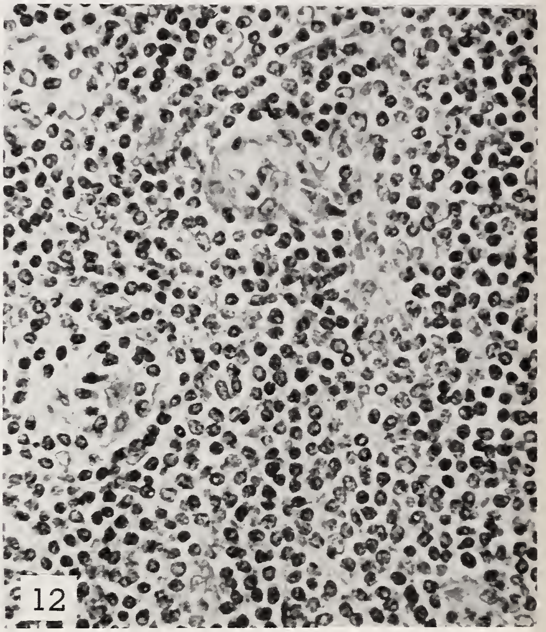
Figure 8.—Angiofollicular lymph node hyperplasia, in which the follicles are in various stages of proliferation or regression. × 9









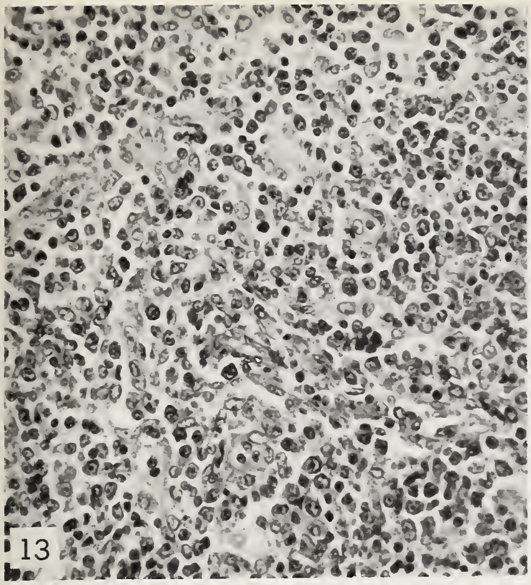


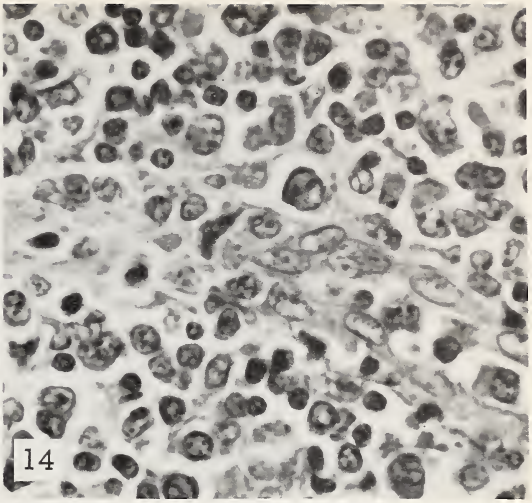
FIGURES 9A AND B.—Higher magnification of 2 follicles from figure 8. One (*A*) is still in proliferative phase, whereas the other (*B*) is in regressive phase, resembling Hassall's capsule. *A*, × 75; *B*, × 200

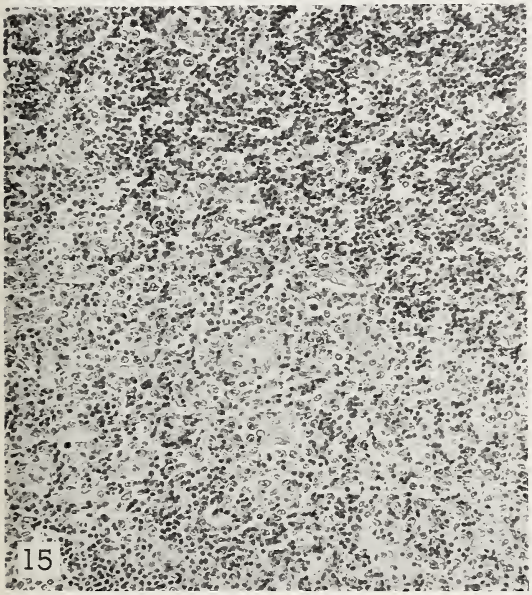
FIGURE 10.—Dermatopathic lymphadenitis. Irregular collections of histiocytes are interspersed with reactive follicles. × 23

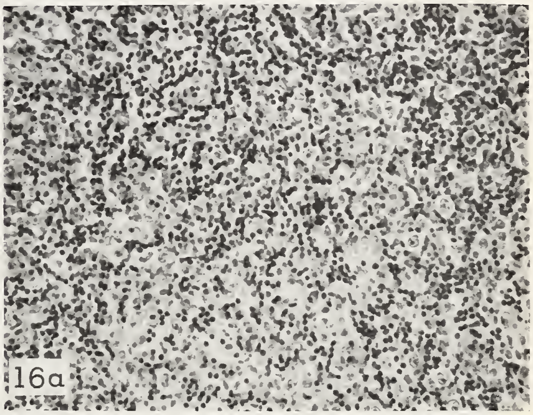
FIGURE 11.—Histiocytes in dermatopathic lymphadenitis. Melanin pigment is finely dispersed, though fat vacuoles are apparent. × 400

FIGURE 12.—Chronic lymphadenitis, in which lymphocytes of varying size are mixed with histiocytes, primitive reticular cells, and plasma cells; because of hyperplasia of endothelial cells, vessel lumina are difficult to visualize. × 400









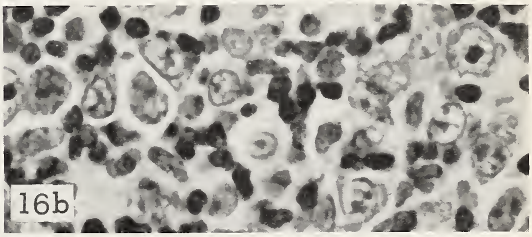


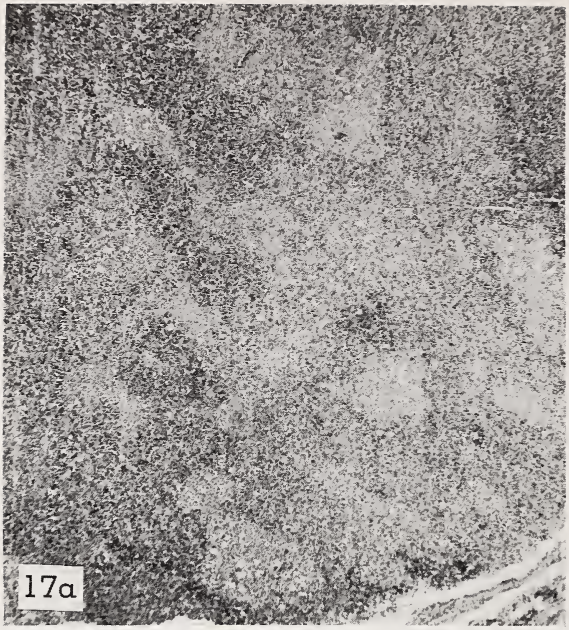
Figure 13.—Infectious mononucleosis. Large, atypical cytoplasmic cells are mixed with normal pulp element. × 200

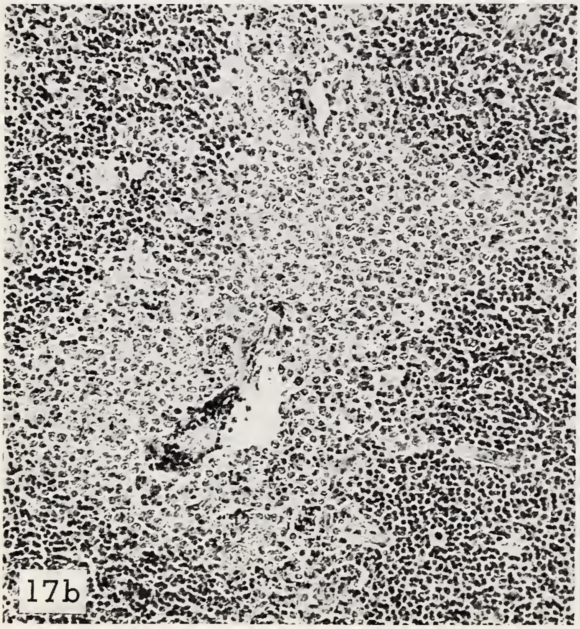
Figure 14.—Infectious mononucleosis. Characteristic cells have large nuclei containing 1 or 2 small nucleoli and have a thin, irregular rim of cytoplasm. × 400

Figure 15.—Postvaccination lymphadenitis. Activated reticulum cells (atypical histiocytes) are visible in pulp. There, too, blood vessels contain hyperplastic endothelial cells. × 100

Figure 16A.—Lymph node draining site of infection in herpes zoster. *Note* similarity to postvaccination lymphadenitis. × 100

Figure 16B.—Higher magnification of figure 16A, showing activated reticulum cells (atypical histiocytes). × 400





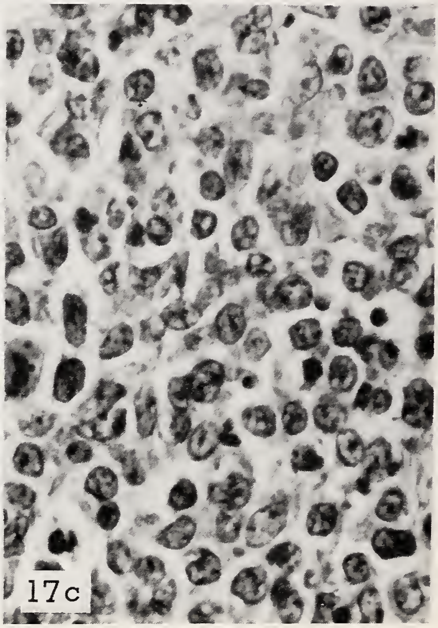
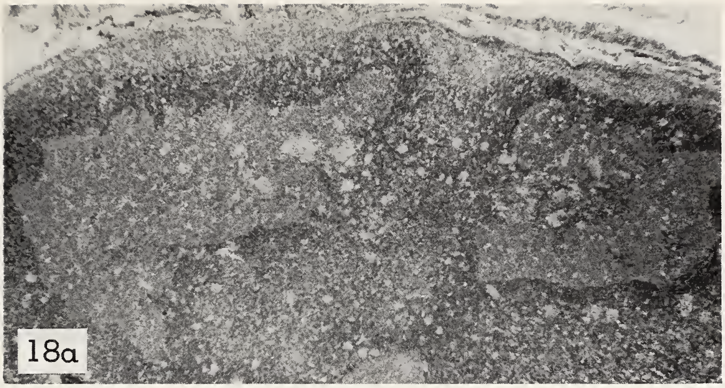
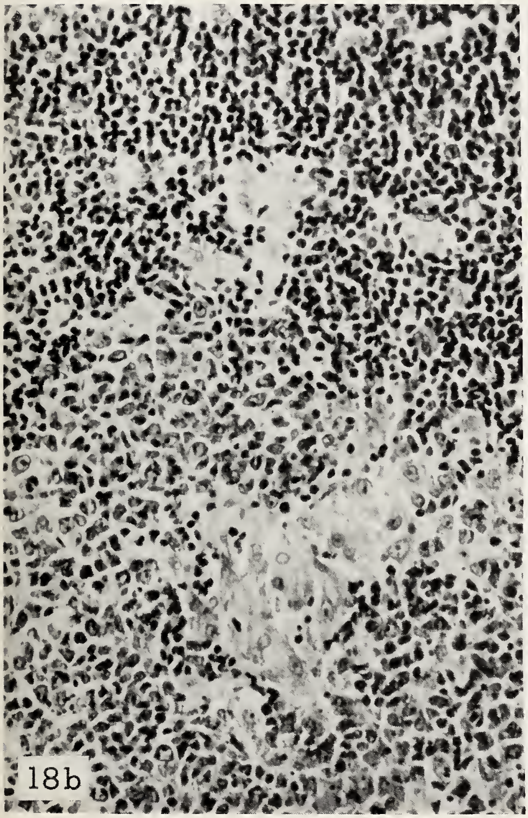


Figure 17A.—Necrotizing lymphadenitis exhibiting hyperplastic reactive follicles and irregular foci of proliferating sinusoidal cells. × 19

Figure 17B.—Higher magnification of one of the foci of proliferating sinusoidal cells in figure 17A. × 100

Figure 17C.—Higher magnification of the sinusoidal histiocytes in figure 17A. Neutrophilic leukocytes were more prominent elsewhere in sinuses. × 400





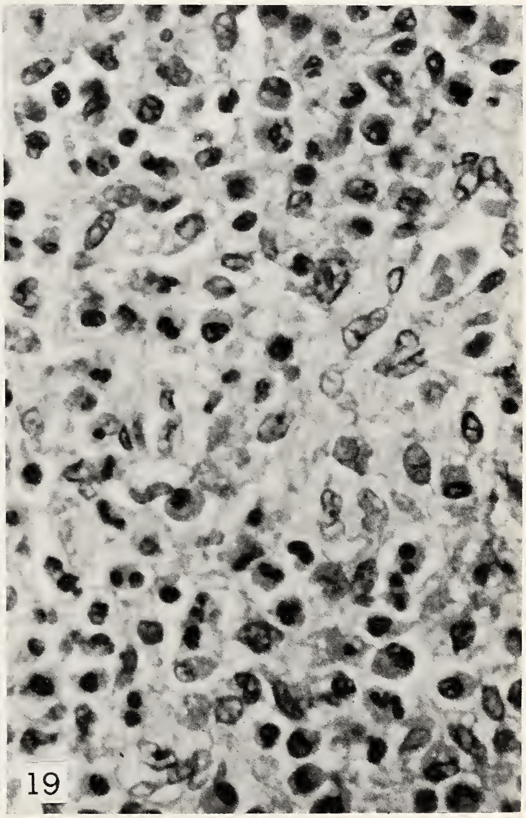


Figure 18A.—Toxoplasmosis of lymph node, in which hyperplasia of all elements is present. Follicles and histiocytic foci, some within follicles, are most prominent at this magnification. × 25

Figure 18B.—Higher magnification of figure 18A, showing foci of histiocytes in reactive follicle and in pulp at its edge. × 400

Figure 19.—Allergic granulomatosis of lymph node, in which histiocytes and eosinophils are normal. This section contains no cells resembling Reed-Sternberg cells. × 400

Non-Neoplastic Hyperplasias of Lymph Nodes of Animals [1, 2]

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AS IN man (*1*), many non-neoplastic diseases of animals are associated with lymphadenopathy. Because most of these have not been specifically defined or characterized, the nodes are described simply as reactive. Veterinary pathologists have little opportunity to examine nodes with various types of non-neoplastic hyperplasias because lymph nodes of animals are rarely removed for biopsy unless they are extremely large. In such cases, they are usually lymphomatous. In this paper a few selected examples of recognized types of non-neoplastic hyperplasias of lymph nodes of animals will be described. Criteria for differentiation of lymphomas from nonmalignant hyperplasias will be outlined and changes associated with early malignant processes will be described.

Well-prepared specimens are required to distinguish neoplastic from benign hyperplastic proliferations. Lymph nodes should be extirpated completely with capsules intact. Specimens should be hemisected to permit rapid penetration of fixative; if very large, nodes should be cut into slices approximately 5 mm thick. Prior to fixation, touch imprints should be made from cut surfaces; these may be dried in air and stained as blood smears. Touch preparations or imprints allow a more critical evaluation of cytologic features than sections of fixed material; the cytologic features are particularly critical to differentiation of lymphoid malignancies from the many types of hyperplasias. Fungal or protozoal agents may be recognized immediately.

In most lymphomas of animals, histologic evidence of malignancy is obvious. However, in occasional lymphomas, particularly in early stages, architectural landmarks as the cortex, medullary cords, and sinusoids may be preserved. Portions of the cortex and medulla may show

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] This study was supported in part by Public Health Service grant FR 000130-04 from the Division of Research Facilities and Resources and training grant GM 00415 from the National Institute of General Medical Sciences, and U.S. Army contract D18-064-ANS-104 (A) Task D.

[3] I wish to acknowledge the technical assistance of Miss Carole Heckner and Mr. Robert Knight and the clerical assistance of Miss Johnnetta Perkins. The photography was by Mr. Peter Lund.

persisting normal cell populations, including those of true germinal centers, and plasma cells. Capsular or trabecular infiltrations may be minimal or absent. In these, multiple sections from different levels of the node should be examined. Imprints are of particular value. Lymphomas of domestic animals nearly always consist of large, poorly differentiated lymphoid cells. Counterparts of well-differentiated lymphosarcomas or chronic lymphocytic leukemia of man are rare in animals. When stained with methyl green-pyronin, imprints of most lymphomas of animals show large, uniformly pyroninophilic cells. In fixed sections these uniform cell populations replace broad areas of cortical tissue. With the possible exception of equine infectious anemia, reactive nodes of animals do not ordinarily show uniform populations of pyroninophilic cells. Imprints of non-neoplastic nodes reveal considerable variation in sizes of cells and in degree of pyroninophilia. Basophilic stains do not emphasize these tinctorial differences.

An important feature which characterizes early neoplasia is the predominance of a uniform population of large lymphoid cells in the peripheral and medullary sinusoids. Careful examination reveals that these are identical to the sheets of cells replacing portions of the cortical pulp. In contrast, the cell populations in sinusoids of reactive nodes are usually very pleomorphic and consist of small and medium-to-large lymphocytes, histiocytes, and perhaps plasma cells. In early lymphomas, the medullary cords frequently consist of normal, well-differentiated plasma cells despite the sinusoidal involvement.

Lymphomas with a follicular or nodular pattern are rare in animals. When they do occur, they are frequently difficult to distinguish from reactive processes. The rarity with which lymphomas of nodular types are encountered in animals may be partially explained by the fact that biopsies are seldom performed in early stages of disease. As in man, neoplastic nodules or follicles must be distinguished from true germinal centers; the same criteria apply for man and animals (*2*). Cells composing neoplastic nodules are generally the same as those of the neoplastic population in interfollicular areas. Although neoplastic nodules may originate from germinal centers, the persisting follicles may be artefactual; persisting non-neoplastic follicles have a familiar mantle of small, uniform lymphocytes surrounding a zone of reticulum cells and large lymphocytes. Mitotic figures are usually no more numerous in neoplastic follicles than in interfollicular areas.

Finally it should be emphasized that there are no specific features of individual cells which distinguish reactive from neoplastic processes. All of the bizarre cytologic changes in cells of lymphomas can result from various immunologic responses. The differentiation must therefore depend on the evaluation of cell populations and their locations, whether nodal or extranodal.

ATYPICAL RETICULUM CELL HYPERPLASIAS

The atypical reticulum cell hyperplasias are a group of idiopathic adenopathies in animals which must be distinguished from lymphomas. They are observed most frequently in dogs and cats. As in man, these hyperplasias may be bizarre immune responses to viral or chemical antigens. In animals, it is rarely possible to identify specific causes by clinical methods. Hypergammaglobulinemia and fever may be present. The adenopathies often regress rapidly following therapy with corticosteroids, or they will often eventually subside without therapy.

Histologically affected lymph nodes may show considerable effacement of architecture, including some cellular infiltration of capsule and trabeculae (fig. 1). The cell population is predominantly histiocytic (fig. 2). Some cells have large bizarre nuclei with prominent nucleoli; occasional giant cells may be present. However, mixed with these suspicious-looking cells are many relatively normal-appearing histiocytes, lymphocytes, plasma cells, and perhaps eosinophils. Islands of small lymphocytes are often preserved in the cortical pulp. This pleomorphism is even more readily appreciated in imprints. The similarity of this hyperplastic process to human Hodgkin's disease may be striking, but typical multinucleated Reed-Sternberg cells are not present. The differentiation of this type of hyperplasia from lymphomas is based primarily on the pleomorphism of the cell population. In man, such a pleomorphic cell population would suggest Hodgkin's disease. In animals, Hodgkin's-type tumors are very rare; it is usually necessary to identify monotonous proliferations of certain types of cells to confirm lymphomas.

ALLERGIC GRANULOMATOSIS

Several unexplained lymphadenopathies of animals including the atypical reticulum cell hyperplasias are probably allergic responses. One specific type observed in cattle apparently results from grazing hairy vetch pasture and, in one report (*3*), affected 6–8% of 800 exposed animals. Mortality in affected animals was almost 50%. The disease was characterized by a diffuse and nodular granulomatous process in many tissues, but was most extensive in lymphoid tissue, adrenal glands, myocardium, kidneys, and thyroid. Lymph node architecture was partially obliterated. The cellular infiltrate consisted of histiocytes, lymphocytes, plasma cells, and eosinophils. The lymphoid cells were mostly large basophilic blastlike cells. The histiocytes showed bizarre nuclei and some giant cell forms. The reaction showed a superficial resemblance to Hodgkin's disease, but Reed-Sternberg cells were not observed.

Biologically, this disease does not behave like a neoplasm. The course is not typically progressive and the mortality is apparently not more than 50%. It does demonstrate that an extensive tumor-like process may result from a naturally occurring hypersensitivity. It further illustrates that antigens capable of inciting this reactivity may be derived from plants, in addition to other chemical or infectious allergins.

EQUINE INFECTIOUS ANEMIA

Equine infectious anemia is a virus disease of horses which is naturally transmitted by mechanical vectors, *e.g.*, biting insects and hypodermic syringes (*4*). Although the disease has been recognized for over a century, the filterable etiologic agent has not yet been isolated and a specific serologic test is not available.

Equine infectious anemia is characterized by periodic episodes of fever, anemia, hypergammaglobulinemia, and generalized lymphoid proliferation. The course is usually progressive, but often there are prolonged latent periods separating acute episodes. Death may occur during the initial illness after an incubation period of 7–60 days or during any exacerbation thereafter. There are many striking clinical and pathologic similarities between the equine disease and certain lymphoproliferative or immunoproliferative diseases of man (*5*). The equine disease is also very similar to Aleutian disease of mink and ferrets.

An interesting significant aspect of this disease is the apparent persistence of the infective agent in the blood of affected horses throughout their lives despite the prolonged periods of latency which may last for months or even years. An unusual type of host-virus interaction must allow this biological behavior, and the virus should probably be considered one of the slow or latent agents.

The tissue alterations produced by this infection are most prominent in the reticuloendothelial system. If death occurs during the initial acute episode, there is usually degeneration or necrosis of lymphocytes, a nonspecific change seen in the acute phase of many virus infections. If an animal survives for several days or weeks, however, the lesions are primarily proliferative in nature. Splenomegaly and lymphadenopathy may be noted at necropsy; histologically there is a prominent increase in medium-to-large basophilic lymphoid cells. This cellular response may partially efface lymph node and splenic architecture, presenting a histologic picture similar to lymphoma (fig. 3). The change is diffuse throughout the cortical pulp and medullary cords of lymph nodes without formation of primary or secondary nodules. There may be infiltration of trabeculae, capsule, and perinodal fat, and sinusoids may contain a uniform population of the same atypical cells. The cells show characteristics of plasma cells, including nuclear eccentricity and clear perinuclear Golgi areas (fig. 4). They look like the hemocytoblasts and plasma cell precursors involved in immune reactions and also the atypical lymphocytes of infectious mononucleosis.

Other tissues, particularly the liver (fig. 5), kidneys, heart, and bone marrow, show infiltrates of similar cells. Hemosiderosis is usually prominent during acute episodes. Fibrinoid necrosis of small muscular arteries and hypercellularity and mesangial thickening of renal glomeruli may also be noted.

The hyperglobulinemia, vascular lesions, and lymphoid proliferation suggest that equine infectious anemia is an immunologic disease. Although the mechanism of production of the anemia is not clear, certain features, as the precipitous fall in hematocrit levels and the prominent hemosiderosis of tissues, suggest a hemolytic process. The cellular infiltrations of bone marrow suggest a myelophthisic or aplastic effect. If hemolysis is involved, the evidence points to an autoantibody with affinity for antigenic determinants on erythrocytes and perhaps other tissue components. Our current understanding of viral infectivity and replication indicates that direct hemolytic action by the virus is unlikely; mature erythrocytes are not likely target cells. In support of an autoimmune hemolytic anemia, Gainer *et al.* (*6*) reported positive direct Coombs' tests, and Moore *et al.* (*7*) using fluorescent methods observed that 20% of erythrocytes of affected horses were coated with an abnormal globulin. If the elevated globulin fraction is functional antibody at all, the persistence of viremia, apparently throughout the life of the animals, would indicate that it does not have specific affinity for viral antigens or that it is for some reason ineffective.

In summary, equine infectious anemia appears to be a naturally occurring, lymphoproliferative or immunoproliferative disease in which cellular infiltrates sometimes resemble those of a neoplastic process. The virus causation should enhance the potential value of this disease as a biomedical model.

OTHER CONDITIONS

Diseases of man and animals which are recognized causes of non-neoplastic lymphadenopathy are metastatic cancer, tuberculosis, brucellosis, toxoplasmosis, histoplasmosis, and other protozoal, fungal, and parasitic infections. Boeck's sarcoid, dermatopathic lymphadenitis, cat-scratch fever, and syphilis, which are well-known causes of lymph node hyperplasias in man, have not been reported in animals.

While reactions of lymphoid tissue to infectious agents tend to be similar in man and in animals, some variations in degree and type of response can be observed in different species. An example is histoplasmosis. In man, most individuals infected with this fungus develop only localized granulomas. Occasionally, however, the organism may become disseminated in the body, causing widespread reticuloendothelial cell hyperplasia in many organs. In dogs, histoplasmosis frequently manifests as a fulminating disseminated disease involving many organs with widespread hyperplasia of cells of the reticuloendothelial system. The characteristic lesion is a marked proliferation of histiocytes with fewer lymphocytes and plasma cells. Often the lymph node architecture is severely effaced with capsular fibrosis and cellular infiltration of the capsule and perinodal fat (fig. 6). Many of the histiocytes have a typical monocytoid appearance in sections, *i.e.*, a distinct indented or folded nucleus (fig. 7). The nuclei have delicate nuclear membranes, very little chromatin, and usually no visible nucleoli. The cytoplasm is broad and eosinophilic. The *Histoplasma capsulatum* organisms are intracellular and may be seen in the cytoplasm of some of the histiocytes.

Finally, some virus and rickettsial animal diseases that may produce lymphoid hyperplasia which is cytologically quite bizarre include infectious bovine rhinotracheitis, canine distemper, canine hepatitis, and a rickettsial disease of dogs termed salmon poisoning. In each of these, specific inclusion bodies or causative organisms can often be identified in sections.

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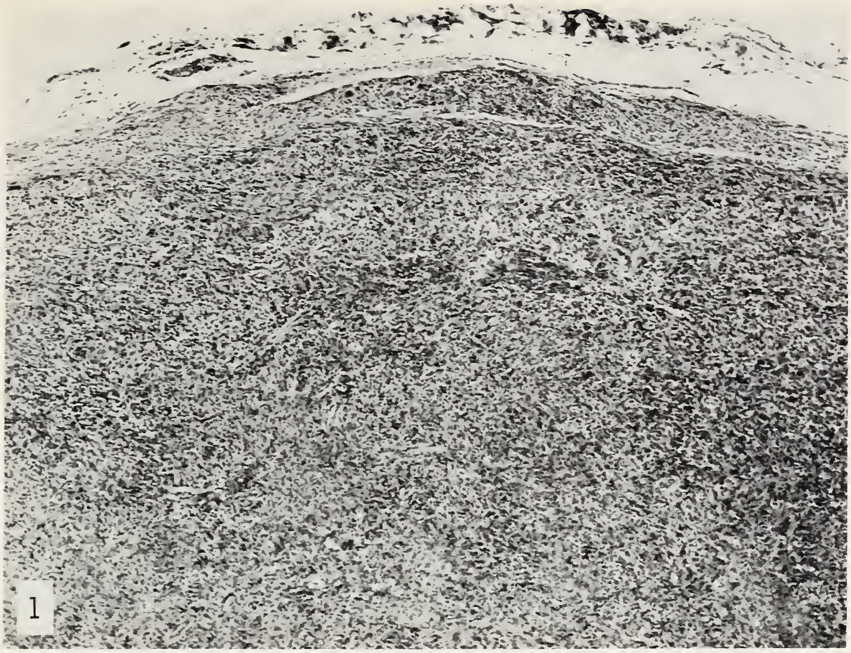


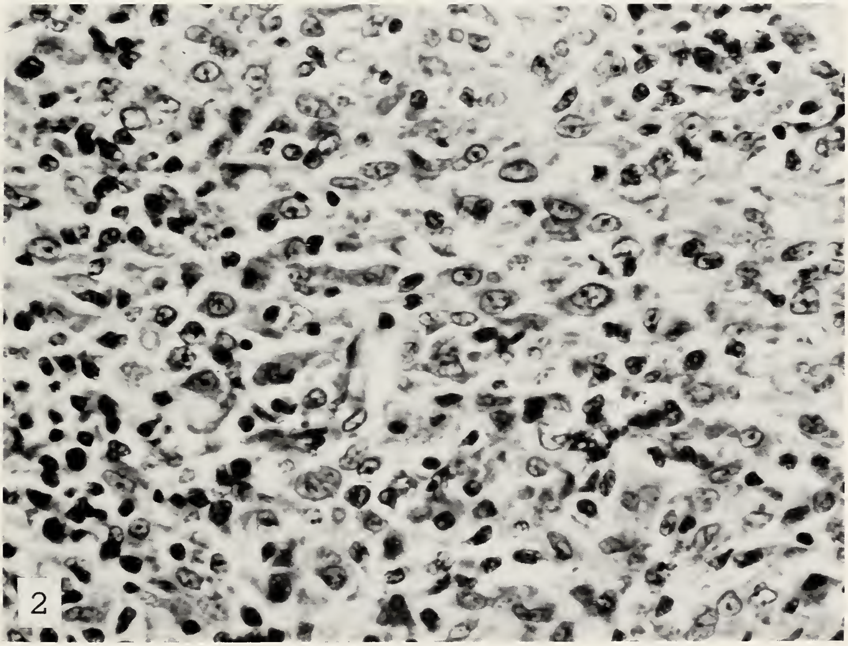
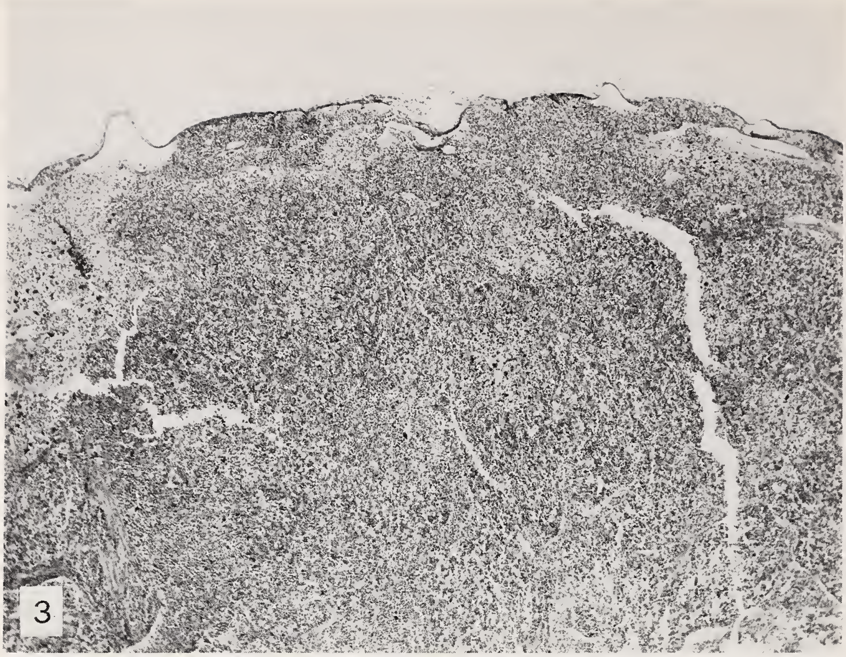


FIGURE 1.—Section of lymph node from cat with atypical reticulum cell hyperplasia. *Note* effacement of nodal architecture and infiltration of capsule and perinodal fat. Hematoxylin and eosin. × 65

FIGURE 2.—Same lymph node as shown in figure 1. Pleomorphic cell population consists of histioctyes, lymphocytes, and a few plasma cells with several large reactive histiocytes (reticulum cells) showing prominent nucleoli and nuclear membranes. Hematoxylin and eosin. × 500



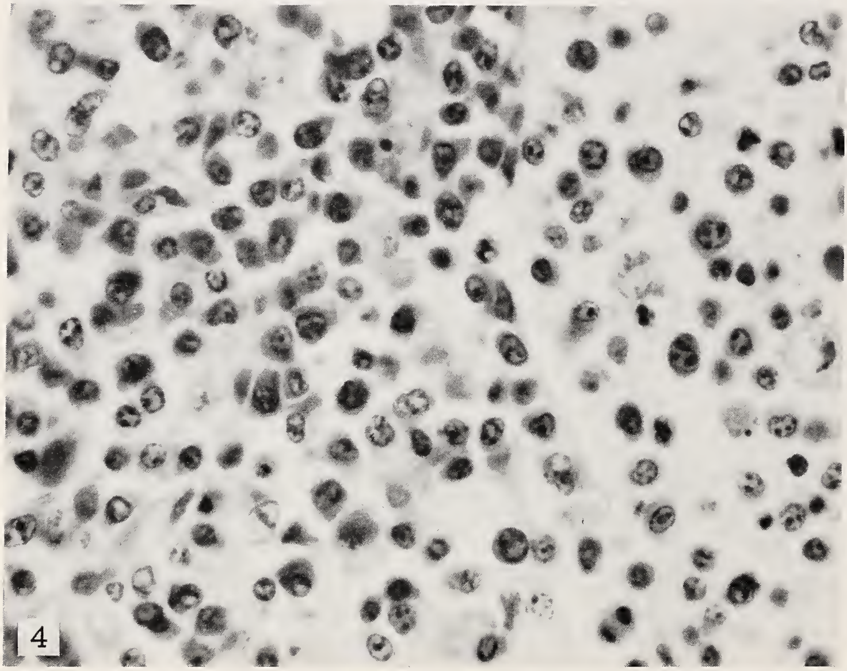
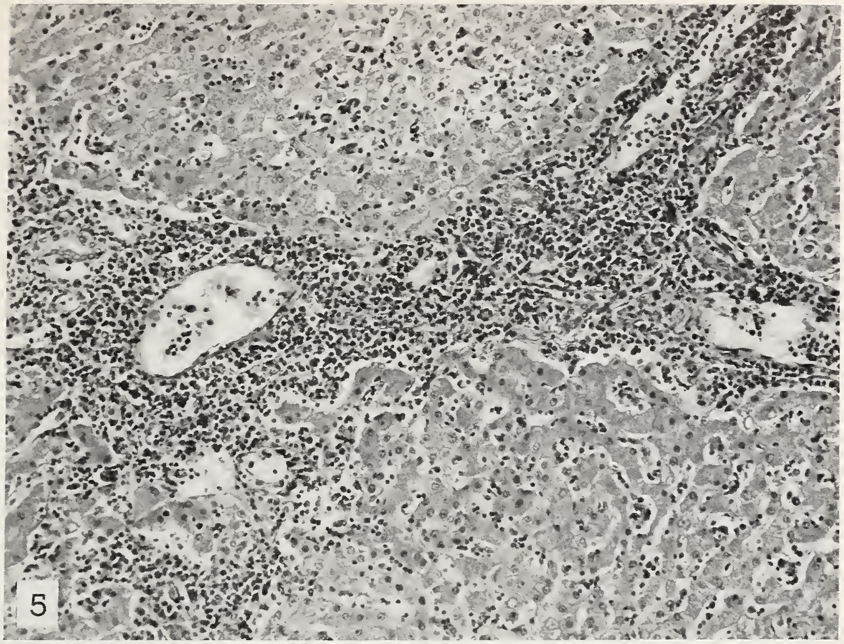


Figure 3.—Section of lymph node from horse with equine infectious anemia showing obliteration of nodal architecture. Hematoxylin and eosin. × 40.

Figure 4.—Same lymph node as shown in figure 3. Predominant cells are large basophilic "plasmocytoid" lymphocytes. Hematoxylin and eosin. × 800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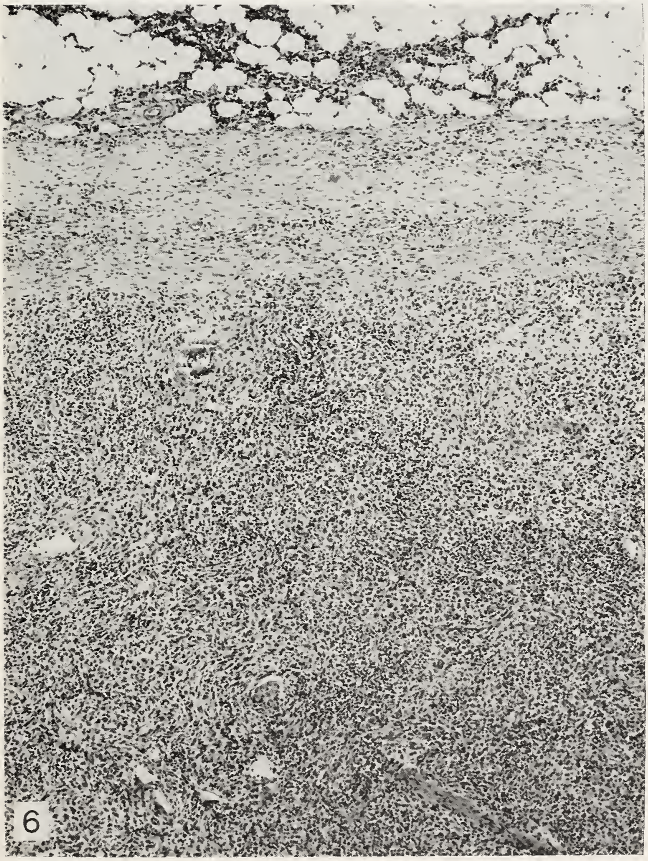
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Figure 5.—Liver section from horse with equine infectious anemia showing severe portal and sinusoidal infiltrates. Hematoxylin and eosin. × 130

Figure 6.—Lymph node section from dog with disseminated histoplasmosis. There is effacement of architecture and cellular invasion of the capsule and perinodal fat. Hematoxylin and eosin. × 65

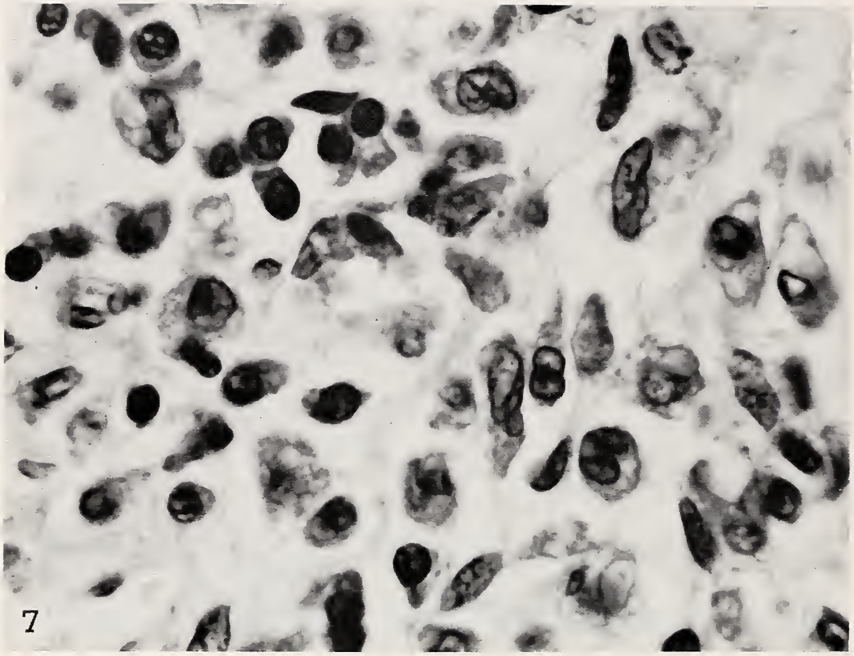

Figure 7.—Same lymph node as shown in figure 6. *Note* large histiocytes with broad cytoplasm and folded or indented nuclei. Large lymphocytes with distinct basophilic cytoplasm are also present. Hematoxylin and eosin. × 1100

Canine Mast Cell Diseases [1]

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SUMMARY

Spontaneous cutaneous and extracutaneous mast cell accumulations are common in the dog but are less frequent in the cat, cow, and man. The *canine mast cell tumor* or *mastocytoma* was previously considered to be primarily a benign cutaneous neoplasm of the dog, with a high affinity for recurrence and extension, but recent evidence indicates that widespread visceral metastasis is likely to occur in most dogs with the tumor. There is some evidence of a multicentric origin, but more clarification of the pathogenesis is needed since cutaneous lesions are usually present when systemic dissemination is recognized. *Mast cell tumors* comprise 7–15% of all canine skin tumors. They occur most frequently between the fifth and tenth years of life. Recently mast cell tumors have been reported to be caused by a virus-like agent. The capacity of mast cell tumors to incapacitate and shorten the lifespan of the hosts must be evaluated.—Nat Cancer Inst Monogr 32: 267–283, 1969.

MORPHOLOGIC FEATURES OF TISSUE MAST CELLS

OUR PRESENT knowledge of the connective tissue mast cell and its involvement in disease processes is based on the efforts of scientists spanning more than a century.

In 1863 von Recklinghausen described and illustrated structural elements in the unstained mesentery of the frog (*1*). His descriptions and drawings clearly include granular cells compatible with *tissue mast cells.* In 1875 Waldeyer recognized, in addition to fibroblasts in connective tissues, granular cells rich in cytoplasm which he referred to as *plasma cells* (*2*). Thus at this early time and perhaps even earlier, mast cells were seen and even described, but were not recognized as a special type of cell.

Ehrlich first recognized (1877) the mast cell as a specific cell type (*3*).

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[1] Based on material presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

In 1879 he proposed the name *Mastzellen* (well-fed cells) and pointed out that these cells possessed cytoplasmic granules that stained with basic dies and that they also altered the dye shade (metachromasia) (*4*). In 1891 Ehrlich and his student Westphal reported that mast cell granules of many species were water-soluble—a fact that should be considered in selecting fixatives and dyes (*5*). Ehrlich observed two conditions in which mast cells increased in numbers—chronic inflammation and the environment of tumors.

Selye credits Unna for the next important discovery about mast cells (*6*). In 1894 Unna reported that the human skin lesion, *urticaria pigmentosa,* consists of dense collections of mast cells (*7*). In 1965 Head (*8*) mentioned that Creighten, in an 1879 paper on subcutaneous cystic tumors of dogs (*9*) in which he described and illustrated 2 tumors that he called *sarcoma myxomatodes,* could have actually been describing mast cell tumors. Creighten's original descriptions and illustrations support Head's opinion (*9*). In 1905 Bashford, Murray, and Cramer reported the first mast cell tumor in a dog (*10*). They described and included an illustration of a round cell tumor consisting of mast cells in the subcutaneous tissue of the leg. In 1942 Bloom provided the first detailed descriptions of the canine mast cell tumor and proposed the term *mastocytoma* for the cutaneous neoplasm (*11*).

What constitutes a tissue mast cell has been debated since Ehrlich first defined the cell as one which possessed metachromatic granules (*4*). This unqualified definition has been difficult to accept for several reasons. Phagocytes often pick up metachromatic material, and granules of mast cells may not be metachromatic or may lose this quality during processing of tissue. To date, no single definition more aptly describes the mast cell than the one proposed by Ehrlich. We can qualify Ehrlich's definition somewhat by limiting it to *connective tissue cells* in which the *basophilic granules normally stain metachromatically* (*6, 12*).

Mast cells have been found in both invertebrates and vertebrates. Each species appears to have its own pattern of cell distribution within the body tissues. Likewise, mast cells of each species have different properties and chemical reactions. In warm-blooded vertebrates, mast cells are observed chiefly in the loose connective tissue surrounding small blood vessels and under epithelial, serous, and synovial membranes. Mast cells are generally sparse in parenchymatous tissues, except in connective tissue trabeculae or capsules of organs. The notable exception to this is the liver of the dog, which normally contains many mast cells (*13*).

The relationship of the tissue mast cell to the blood basophil (or blood mast cell) is not understood, but most authors agree with Ehrlich's original contention that these two cells are genetically distinct. Furthermore, Sagher and Even-Paz pointed out that these 2 types of cells can be distinguished both morphologically and cytochemically (*12*). Nevertheless, an inverse numerical relationship between tissue mast cells and blood basophils has been noted in several species (*14, 15*).

The relationship between tissue mast cells and eosinophils has been studied with much interest to attempt to explain the appearance at times of relatively large numbers of eosinophilic leukocytes associated with mast cells in some pathologic conditions. Many investigators believe that eosinophils are concerned with detoxification and disposal of histamine, including that released by tissue mast cells (*12*). Hottendorf and Nielsen, however, studied collagen necrosis in canine mastocytomas and noted large numbers of eosinophils; they suggested that an autoimmune mechanism may be involved (*16*).

Tissue mast cells are normally round, oval, polygonal, or pyriform, and more rarely spindle-shaped. The cell membranes are usually recognizable, but not always. The nuclei are typically round or oval but can be indented. They tend to vary in size, and binucleate and multinucleate forms are sometimes observed. One or two nucleoli may be present. Mitotic figures are rare. The cytoplasmic granules vary from large to minute rounded bodies but tend to be uniform in size in a given cell. They may be so few as to be almost imperceptible or so abundant that the nuclei are difficult to discern. Occasionally cytoplasmic vacuoles are observed. Electron microscopic studies of the morphologic structure of tissue mast cells have been accomplished by several investigators working with animal and human tissues (*17–19*). In general, normal mast cells from diverse tissues and species appear similar (*20, 21*). The characteristic round-to-oval cytoplasmic granules tend to be prominent and electron-dense. The internal structure of the granules as seen by electron microscopy is usually indistinct and has been described as finely granular, reticular, vacuolated, and filamentous. Possible explanations for differences in appearance of the internal structure have included the state of maturity of the granules, a phase in function, or technical problems in preparing the tissues for study (*12*).

The recognition of tissue mast cells in a pathologic process depends largely on their characteristic metachromatic granules and less on their size or general morphology. The staining reaction of the granules is at times unpredictable, and the granules do not always stain with dyes considered to be specific or selective. These facts should be considered when evaluating histologic or histochemical findings. Sometimes normal tissue mast cells can be found in tissues adjacent to a pathologic process. In such cases these cells serve as a welcome control for the stain used.

In hematoxylin-and-eosin-stained sections from dogs, the granules of mast cells are usually a light red and rarely blue. They stain purple to red with toluidine blue, methylene blue, azure A, and certain other dyes having little or no red constituents. We of the Veterinary Pathology Division use the Giemsa stain and Luna's mast cell stain routinely for animal tissues and achieve satisfactory results (*22*). It is not within the scope of this paper to cover all aspects of metachromatic dyes. In 1935 Lison pointed out that the term *metachromatic staining* can be correctly applied only when a single pure dye is used (*23*, *24*). Thus stains such as Giemsa which are composed of a mixture of dyes are more correctly termed *differential* stains. In practice, it is most difficult to comply with a requirement for using pure dyes. Toluidine blue, azure A, and thionine possess definite metachromatic properties, and nonmetachromatic properties are usually minimal whereas dye mixtures of the Romanovsky type, such as Giemsa and Wright stains, contain measured quantities of methylene blue and variable amounts of its lower metachromatic homologs azure A or B (*25*). Giemsa stains mast cell granules bluish violet to reddish purple. Because one or more metachromatic homologs may be present in the Giemsa stain, and in inconstant concentrations, researchers often avoid using this stain for studying mast cells. The Luna mast cell stain is nonmetachromatic and colors the mast cell granule deep purple with aldehyde-fuchsin. Halmi and Davies compared the reactivity of several tissues to aldehyde-fuchsin, toluidine blue O, and periodic acid-Schiff (PAS) (*26*). They found most metachromatic components were positive also to aldehyde-fuchsin, and fixatives that may alter metachromasia did not necessarily diminish their affinity for aldehyde-fuchsin. Likewise they found that many orthochromatic, PAS-positive structures stained with aldehyde-fuchsin. We have found that the Luna mast cell stain works well on tissues of animals and particularly the granules of the mast cells of dogs. The PAS reaction is often used to stain mast cell granules. The granules tend to stain irregularly with PAS, and granules of some cells may stain while others do not (*27*). It has not been definitely established whether this variability is due to biochemical differences within granules or variations in technique, or both. Sagher and Even-Paz pointed out that PAS reactivity (magenta color) and metachromatic staining are often inversely related (*12*). Hottendorf and Nielsen, studying 300 mast cell tumors of the dog, reported that mature granules were either PAS positive or PAS negative (*28*). Granules of anaplastic mast cells stained faintly PAS positive and were interpreted as being the least mature.

Fixation of tissues containing mast cells has been a problem, and investigators have tended to disagree on the methods, partly because material has been obtained from different species of animals, and techniques of fixation and staining vary in different laboratories. The water solubility of the granular substances is one of the most striking differences between mast cells of various species of animals (*12*).

Most tissue specimens are submitted to our laboratory in 10% formalin. In our experience, formalin appears to be a satisfactory fixative for tissue mast cells of cats, dogs, and cattle. The granules of mast cells of dogs and cats are relatively insoluble in water, while those of cattle are reported to be moderately soluble (*6*, *29*). Saal and Riek reported formalin to be an acceptable but not an ideal fixative for mast cells of bovine skin (*30*).

PATHOLOGIC CONDITIONS INVOLVING MAST CELLS

Recently the term *mastocytosis* has been introduced to cover the broad spectrum of pathologic conditions of man and animals that involves mast cells (*31*). It is not clear whether tissue mast cell diseases are comparable in all species. In animals, particularly dogs, both neoplastic and reactive increases in tissue mast cells are recognized. Mast cell diseases of man and dogs are similar (*32*), but mast cell tumors are relatively more frequent in dogs. Mast cell neoplasms of the skin of dogs have been called *mast cell tumors, mast cell sarcomas, or mastocytomas,* and the term *mastocytosis* is used primarily for the systemic reactive mast cell proliferations (*33*).

The term *mastocytosis* designates all pathologic conditions in man characterized by proliferations of tissue mast cells (*6, 12*). Most commonly, human mastocytoses affect the skin, but the process may also involve many organs when it manifests as a systemic disease. The skin lesion *urticaria pigmentosa* is the most common form of mastocytosis in man (*6, 12*).

The *mastocytoma* is considered to be one of the most common mesenchymal tumors of the dog (*28, 33*). Interesting but limited data are available on the age and sex of afflicted dogs. Apparently no age group is completely free from mastocytomas. One recent report includes a dog 7 months old and one 18 years of age (*28*). Three recent and relatively large studies indicate the mean age to be approximately 8 years. Most cases occur between the 5th and 10th years. There is no obvious predisposition for either sex (*28, 34, 35*). The boxer is generally considered to be the breed in which mast cell tumors occur most frequently (*35, 36*).

On gross examination, cutaneous mast cell tumors of dogs usually appear well circumscribed but occasionally are poorly demarcated and involve the dermis. They range in diameter from 1 cm to over 10 cm, with a slightly elevated center (fig. 1). The overlying epithelium is ulcerated in 9–30% of the cutaneous neoplasms (*28, 34, 37*). Mast cell tumors occur most frequently on the hind quarters and genitalia, but they may occur on the head and anterior portions of the body (*28, 33, 34, 37*). Orkin (*31*) and Emerson (*38*) reported that the predilection for the rear quarters and genitalia may be due to a relatively high normal mast cell population at these sites. The overlying epithelium may have a normal hair coat, or may have sparse hair or none. The tumors are usually firm but may be soft. On cut surface they are typically white, but may be tan or gray with red and orange areas. While the tumors may appear to be well encapsulated on gross examination, a capsule is seldom observed on microscopic examination. Tumors are multiple in 11–14.3% of dogs (*28, 34*). Tumors may be present from 2 weeks to several years. The clinical history often includes remarks that the tumor has been present for a long time and has suddenly started growing during the preceding few weeks. Recurrence at the site of surgical removal is common, but reported figures vary from 10–50%. Recurrences have been reported as early as 2 weeks after excision and as late as 2 years. Follow-up information is difficult to obtain, and much of it is very limited (*28*, *34–37*). Sometimes the recurrence is actually a metastatic lesion in a regional lymph node close to the original cutaneous site. Head reported that 80% of the dogs he examined by biopsy or necropsy had involvement of lymph nodes (*34*). Hottendorf and Nielsen recently summarized necropsy reports of dogs with mastocytomas in 21 published papers (*39*). They reported that 76% of the dogs had accumulations of mast cells in regional lymph nodes, the spleen was involved in 46%, and the liver in 41%. In 15% the lungs had greater than normal numbers of mast cells. Also involved were the kidneys in 9%, the heart in 4%, and the bone marrow in 9% of dogs examined. In their own report of 29 necropsies, these investigators found that 28 dogs (96%) had accumulations of mast cells in regional lymph nodes draining the site. Of the 29 dogs, 23 (79%) had increased numbers of mast cells in the spleen. Eighteen livers (62%) were involved, and 11 (38%) dogs had accumulations of mast cells in the kidneys. Of the 29 dogs, 10 (34%) had increased numbers of mast cells in the lungs. Of the 29 hearts, 6 (21%) contained foci of mast cells (*39*). Weiss and Hanichen reported involvement of internal organs in 80–90% of dogs with cutaneous mast cell tumors (*35*). They found that the draining lymph nodes, spleen, liver, and lungs were involved with decreasing frequency in the order listed. They occasionally detected mast cell foci (mast cell reticulosis) in kidneys, bone marrow, and heart muscles.

The microscopic appearance of the cutaneous canine mastocytoma has been described by several capable authors (*28*, *32*, *34*, *37*, *39*). Head (*34*) classified the tumors into four types. Hottendorf and Nielsen (*28*) classified them into three types, ranging from very anaplastic neoplasms to neoplasms composed of mature-appearing cells (fig. 2). Weiss classified tumors of his series according to the criteria established by Head (*34*) and Larsson (*40*). In general, the literature does not agree as to gross and microscopic indications of malignancy. Typically, mastocytomas involve the dermis principally, with a tendency to invest but not to invade adnexal structures or overlying epithelium (fig. 3). Extension into the subcutaneous tissues, including skeletal muscle, is common. Small, slow-growing neoplasms are composed primarily of mature-appearing mast cells with numerous large cytoplasmic granules. Mitotic figures are rare, and the cells tend to be arranged in rows (figs. 4, 5, and 6) and nests, or may be diffuse. Eosinophils may be numerous or virtually absent. Collagen fibers are prominent in the mature-appearing neoplasms and are believed to be pre-existing (fig. 7). Plasma cells, lymphocytes, and even neutrophils may be observed within the neoplasm.

In the more rapidly growing neoplasms, numerous mature-appearing mast cells may be present, but many cells may have small, almost dustlike, or indistinct granules, which may or may not stain metachromatically. In some cells granules are sparse. Occasionally mitotic figures are observed, and collagen may be either virtually absent or plentiful. If the overlying epithelium is ulcerated, an inflammatory or even granulomatous component may be present. Hyalinization of walls of small vessels and edema are sometimes observed in tumors with prominent stroma. Occasionally fragments of collagen may be surrounded by eosinophilic leukocytes (fig. 8) (*16*). Mast cells may vary in size and large multinucleate forms are rarely seen (figs. 9–13). Tumor mast cells vary not only in size of granules but also in shape, largely from adapting to the surrounding tissues. Occasionally cytoplasmic vacuoles are present; these may be artifacts caused by chemical or physical damage to the cells. Paff and Bloom (*41*) regarded the vacuoles as an effect of aging or as being due to secretion of histamine or heparin (*42*). Intranuclear and cytoplasmic inclusions have been described by Weiss and Bloom. These range from cytoplasmic crystalloid structures to intranuclear inclusions that are PAS-positive and may be so large as to almost fill a nucleus and be surrounded only by a small but distinct halo (*11*, *33*). Weiss studied the histochemistry of 4 canine mast cell tumors with intranuclear PAS-positive inclusions. He found similar intracytoplasmic accumulations of the same material in about one-third of all his canine and feline mast cell tumors. He believes the inclusions consist of glycogen and are an expression of abnormal carbohydrate metabolism rather than indicating a possible viral etiology of mast cell tumors (*43*).

In the proliferative diseases of man associated with dysproteinemia, including multiple myeloma and the primary macroglobulinemia of Waldenström, intranuclear and cytoplasmic PAS-positive inclusions are observed in plasma cells and in the so-called intermediate forms between lymphocytes and plasma cells. The cytoplasmic inclusions resemble Russell bodies. Immunofluorescent techniques have been used to demonstrate that these inclusions are glycoproteins (*44*, *45*). The intranuclear inclusions of these cells are similar to those observed in mast cell tumors of the dog. This similarity is interesting in view of the possible interrelationship between plasma cells and mast cells, as suggested by Hottendorf, Nielsen, and Kenyon (*46*) (fig. 14).

Lombard *et al.* reported that canine mast cell tumors are caused by a viral agent (*47*, *48*). In 1968, Rickard and Post reported a transmissible mast cell leukemia (*49*).

COMMENTS

Acceptable criteria for malignancy, based on gross and microscopic morphologic features, have not been established for canine mast cell tumors. Invasive growth, recurrence after surgical removal, and metastases occur with relatively high frequency. Multiple tumors, or a tendency to multicentricity, is particularly characteristic of highly anaplastic mast cell tumors. The high incidence of mast cell "reticulosis," observed in

70–90% of necropsied dogs with mastocytomas, is not completely understood. Hottendorf and Nielsen described typical metastatic lesions in the spleen and lymph nodes in a perifollicular pattern suggesting multicentric origin of neoplastic mast cells (*39*). *Mast cell leukemia* may occur during terminal phases of canine mastocytosis (*33*, *50*). It is interesting that all reports of extracutaneous mast cell proliferations have been in dogs with cutaneous lesions, with the exception of a beagle described by Rickard and Post (*49*). The relationship of the extracutaneous proliferations to the cutaneous mastocytoma is not clear. Hottendorf and Nielsen (*28*) evaluated the potential of mastocytomas for shortening the life of the host. In the study by Cohen *et al.* (*51*), in which only 9.7% of dogs of the normal canine population were 10 years or older, necropsied dogs with mastocytomas averaged 10.5 years, which indicates that the risk that a mastocytoma will shorten the lifespan of the host is minimal.

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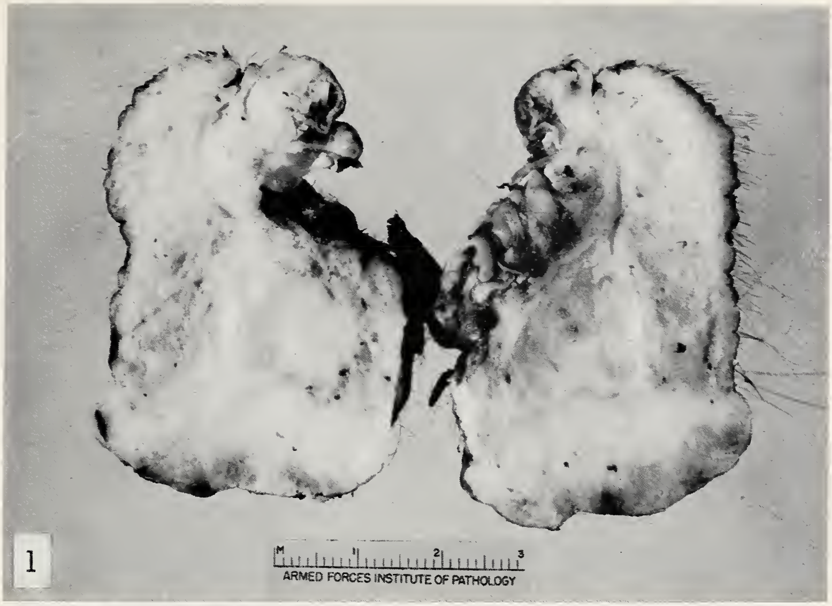
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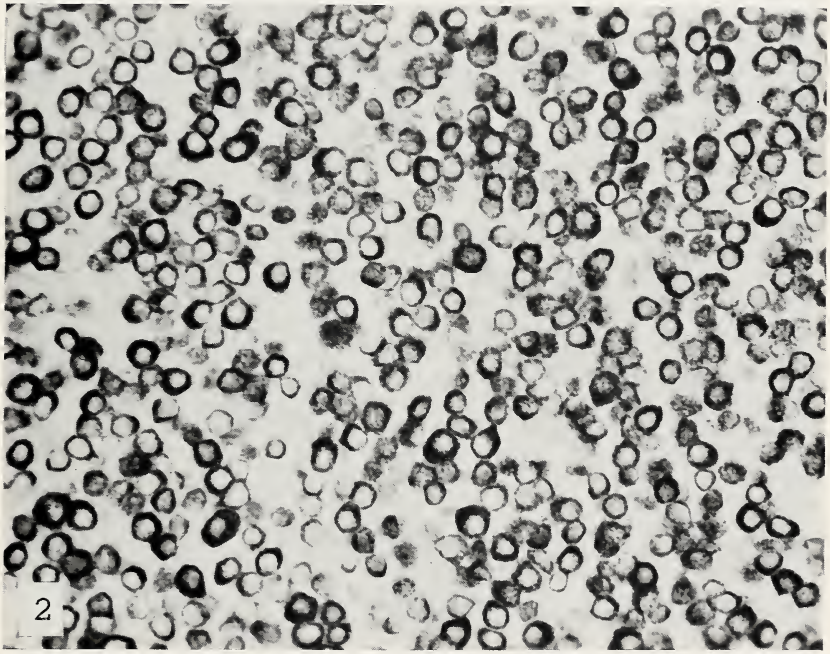
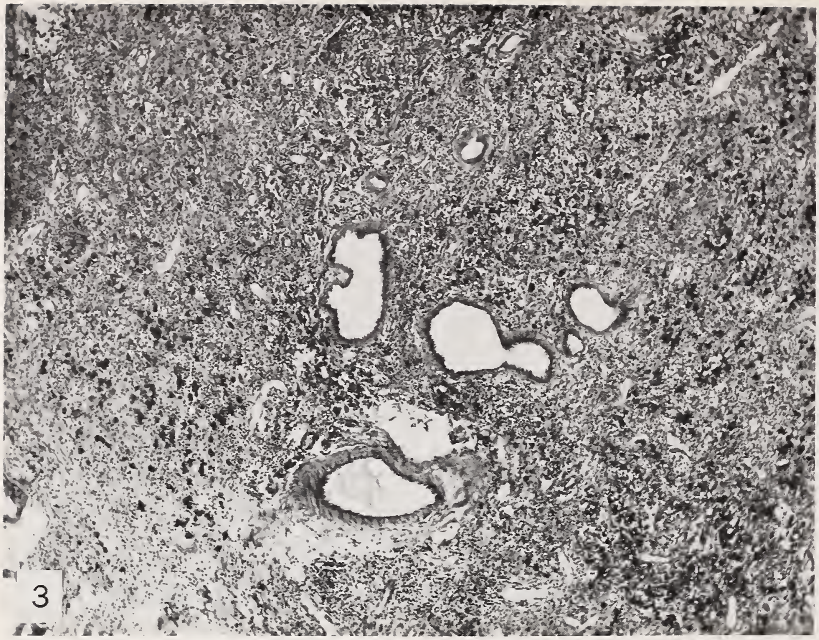


FIGURE 1.—Gross photograph of a mastocytoma which originated in the scrotum of a 13-year-old male spitz dog. Mastocytoma is an expanding nonencapsulated mass involving dermis and subcutaneous tissue. AFIP negative 56-6265.

FIGURE 2.—Giemsa-stained section of a well-differentiated mastocytoma. *Note* prominent cytoplasmic granules. AFIP negative 53-8321. × 485



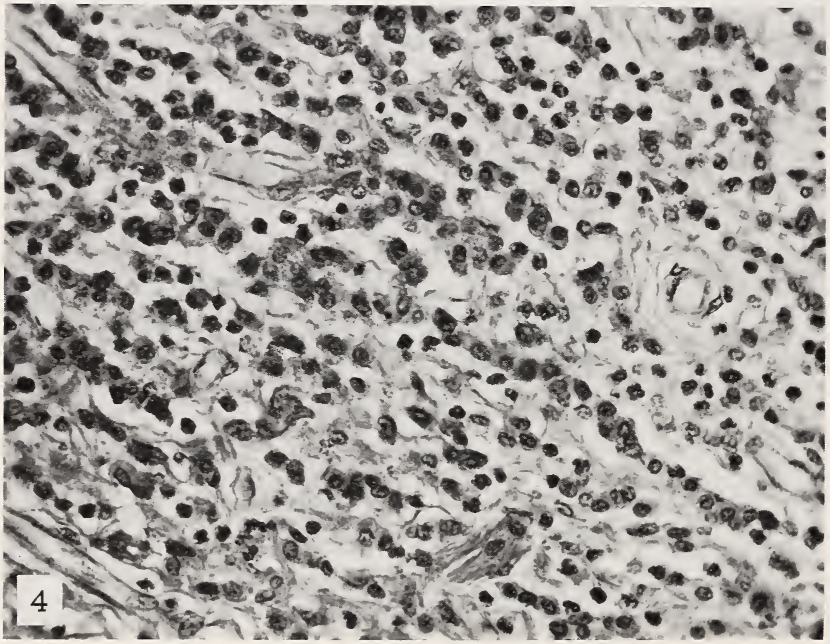
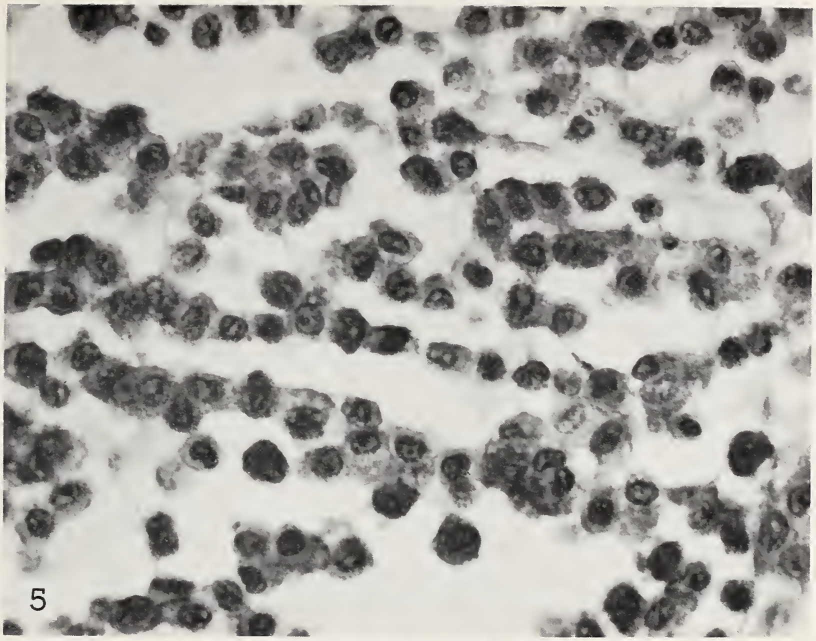


Figure 3.—Mastocytoma investing dilated apocrine gland ducts. Giemsa. AFIP negative 68-8223. × 55

Figure 4.—Mastocytoma showing cells lined up in rows. Mature eosinophils are numerous. Hematoxylin and eosin. AFIP negative 68-8217. × 350



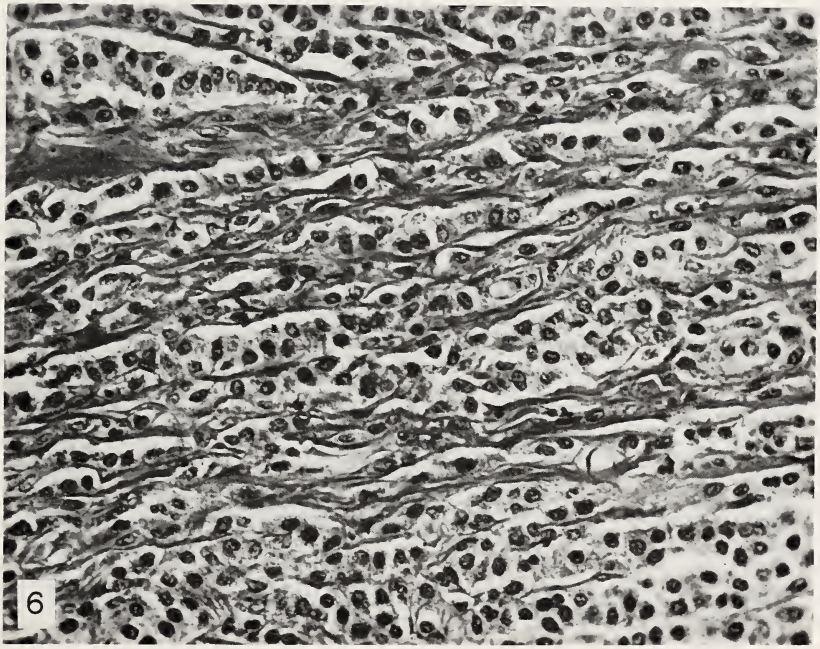
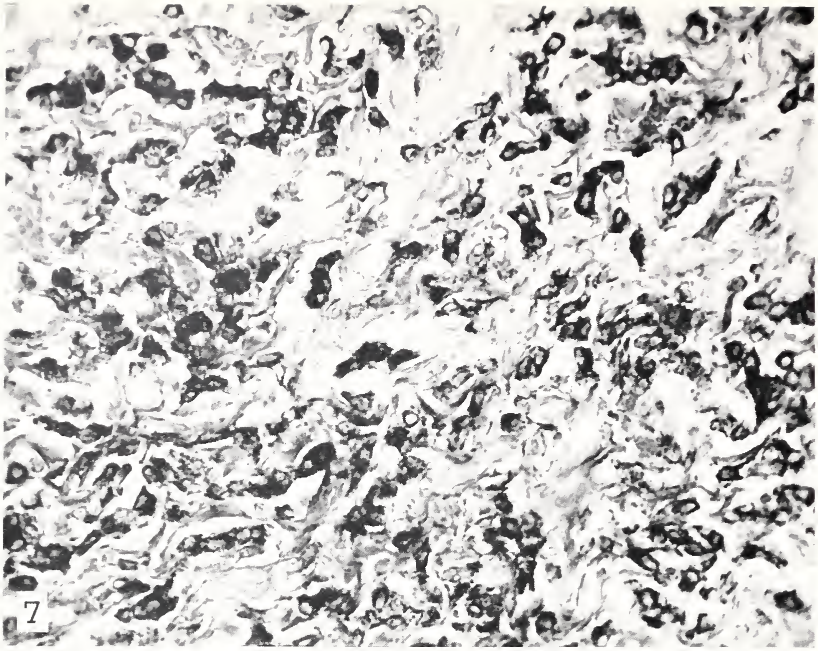


Figure 5.—Higher magnification of tumor shown in figure 4. AFIP negative 68-8850. × 700

Figure 6.—Mastocytoma with cells lining up in rows along a heavy collagen component. Hematoxylin and eosin. AFIP negative 68-8216. × 350



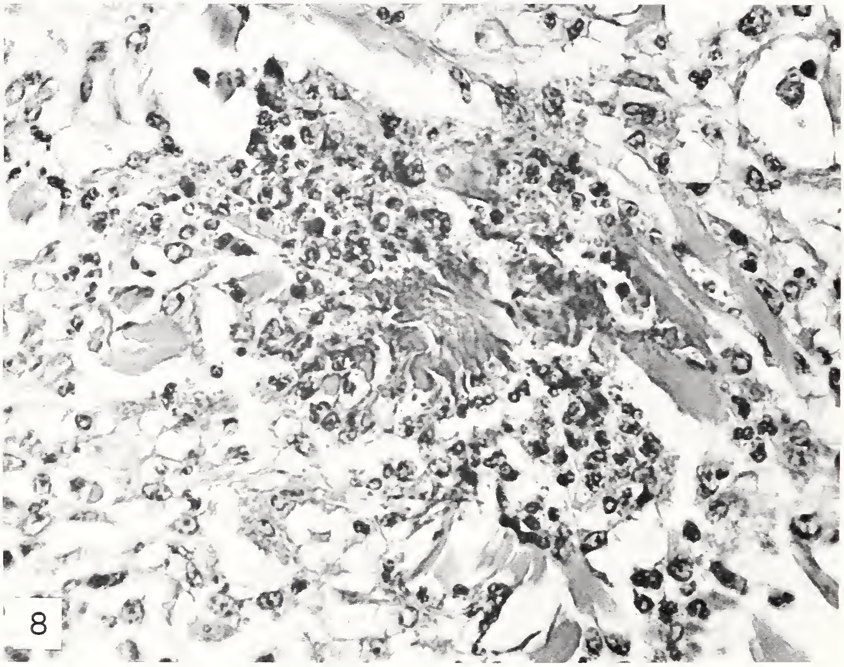
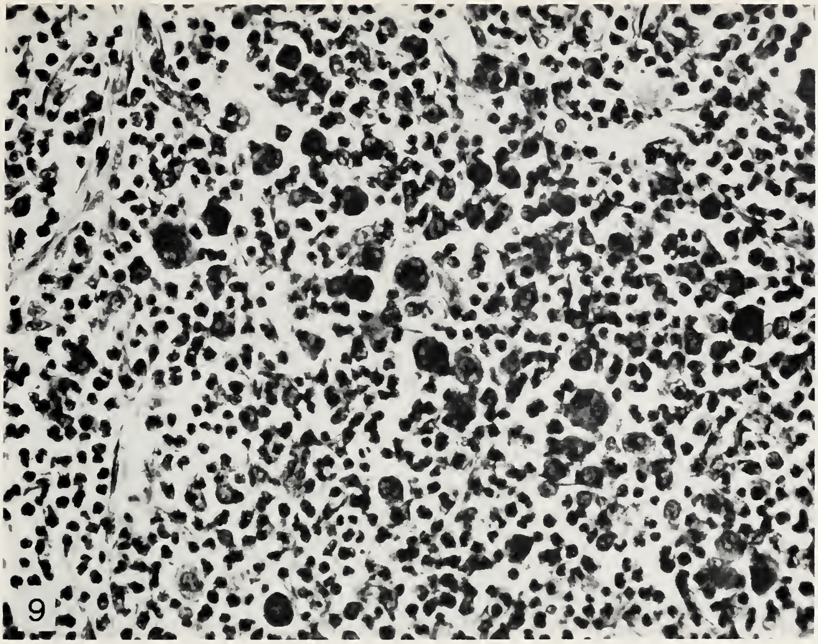


FIGURE 7.—Mastocytoma composed primarily of mature cells with heavy component of collagen. Giemsa. AFIP negative 68-8219. × 350

FIGURE 8.—Mastocytoma with necrosis of collagen. *Note* fragments of collagen surrounded by eosinophils. Hematoxylin and eosin. AFIP negative 68-8211. × 350



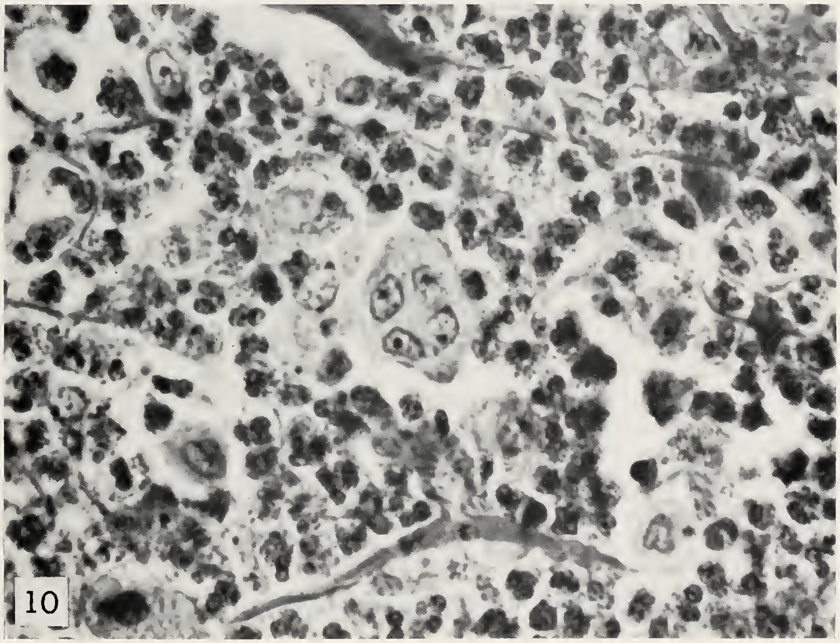
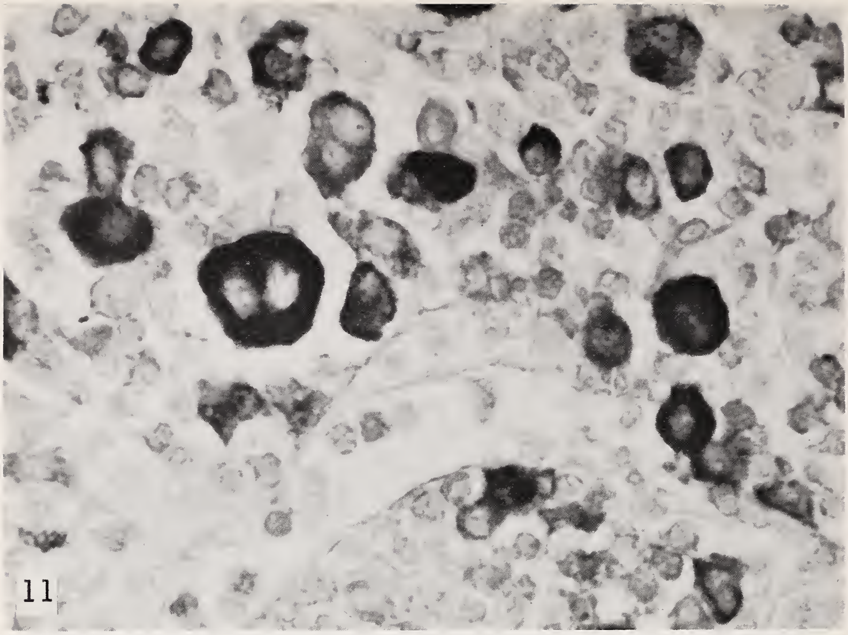


FIGURE 9.—Mastocytoma with multinucleated cells and many eosinophils. Hematoxylin and eosin. AFIP negative 68-8220. × 350

FIGURE 10.—Mastocytoma with a large multinucleated cell surrounded by eosinophilic leukocytes. Hematoxylin and eosin. AFIP negative 68-8880. × 800



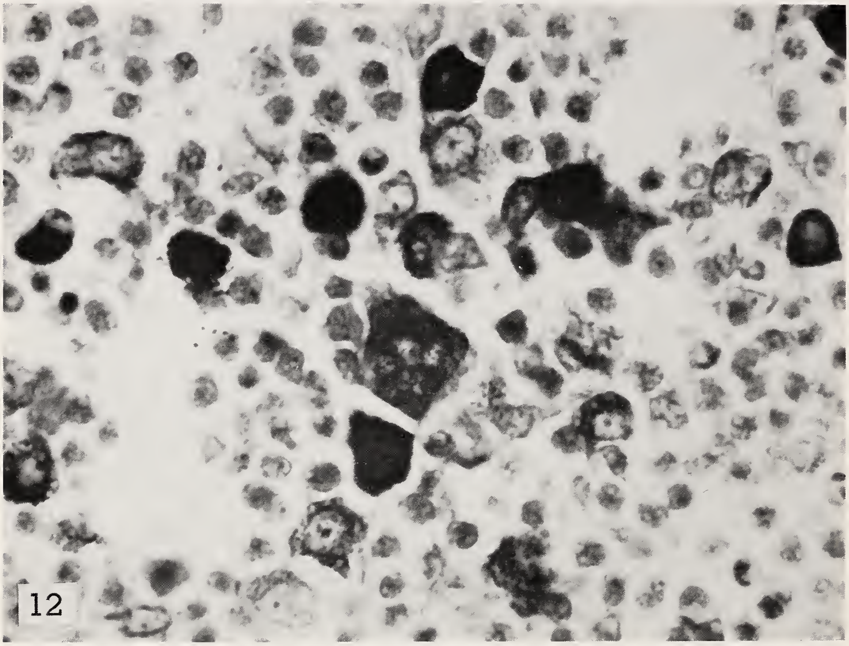
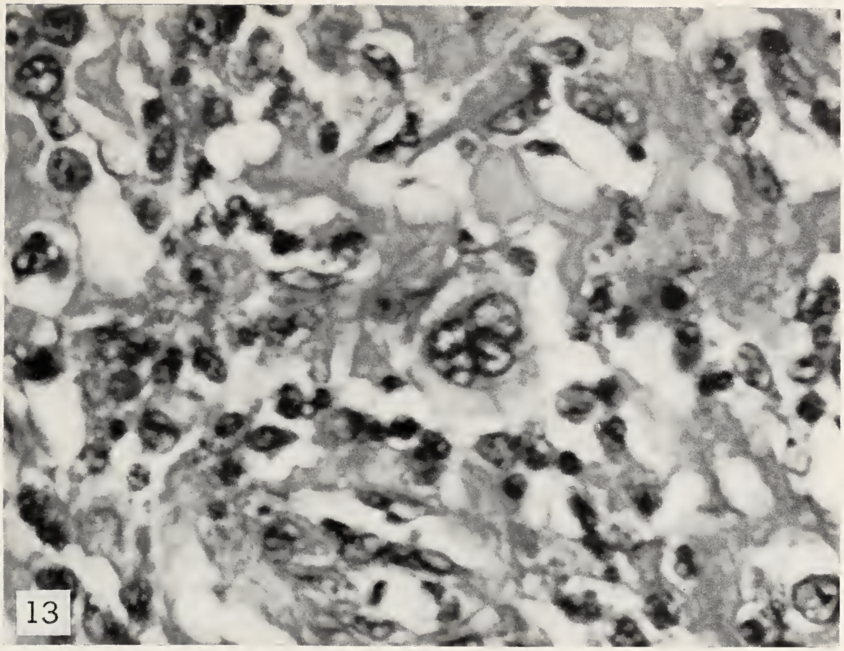


Figure 11.—Mastocytoma with binucleate cells. Granules of mast cells are small. Luna mast cell stain. AFIP negative 68-8879. × 800

Figure 12.—Cutaneous mastocytoma with multinucleated giant cell. *Note* that granules almost obscure nucleus. Giemsa. AFIP negative 68-8881. × 800



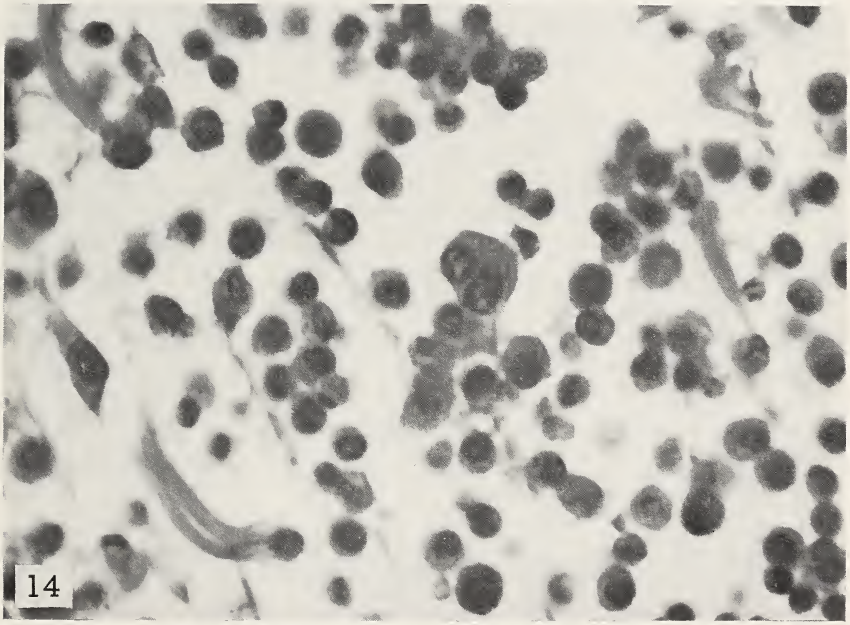


FIGURE 13.—Multinucleated mast cell in a pleomorphic mastocytoma removed from the posterior area of scrotum of a 5-year-old male Boston terrier. An unidentified neoplasm was reported to have been removed from the same area 2 or 3 years before. AFIP negative 68-8852. × 700

FIGURE 14.—Area of a mastocytoma which illustrates similarity of mast cells to plasma cells. Hematoxylin and eosin. AFIP negative 68-8885. × 800

Mast Cell Neoplasms of Mice[1]

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THERE ARE several puzzling features about mast cells in mice that I wish to emphasize. I believe the mouse has been neglected in studies of mast cells. I once complained about this to Dr. Jacques Padawer who told me that he would like to use them, but that all mice were tied up with the chemotherapy program.

My observations of mast cells and mast cell tumors of mice have been published elsewhere (*1–3*). For this Symposium I wish to stress some features of mast cells of mice that may be of interest to comparative pathologists.

A great deal has been said concerning the location of mast cells along blood vessels, but mast cells are also observed in other locations, including areas surrounding mammary ducts and in spleens of mice of some strains.

We have noted a curious difference in distribution of mast cells in spleens of different inbred strains of mice. Spleens of mice of the inbred strain, I, stained with toluidine blue, contain almost no mast cells, whereas spleens of mice of another inbred strain, A, have innumerable mast cells. In strain A mice, mast cells increase as the animal ages and are especially numerous in old male mice. We have counted up to 2,000 or more in a single longitudinal section.

Another curious phenomenon involving mast cells of mice is observed in adrenal glands. In some strains, fibrosis develops just beneath the capsule. Ordinarily mast cells are never seen in adrenal glands of mice, but they become numerous in these fibrotic areas. A similar appearance of mast cells may occur in a damaged fibrotic kidney. The mouse is said to have no circulating blood basophils, and this has certainly been our experience with mice. These observations indicate that the mast cells in the adrenal gland must have been formed at the sites of fibrosis rather than having been transported by the blood.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Mast cells proliferate in the skin of mice after painting with 3-methylcholanthrene. This proliferation has been studied by Cramer and Simpson (*4*) and may be a neoplastic proliferation in response to the carcinogen. However, on attempts at transplantation, mast cell tumors have not developed, perhaps because other cells in the area have a greater potential for growth.

A curious phenomenon we have seen a number of times occurs in association with a mouse hepatoma. The area of the hepatoma is infiltrated with apparently normal mast cells within the sinusoids. Normal mast cell infiltration of tumors has also been reported in rats, and even during transplantation the mast cell infiltrates remain. The best paper on this subject was published by Bali and Furth (*5*) who worked with transplantable splenic tumors in mice.

Some years before this, Dr. Jacob Furth and I discussed mast cell tumors. Each of us hoped to get a murine mast cell tumor, and we set up a friendly contest. Quite by chance we both obtained a transplantable mast cell tumor at about the same time (*1*, *5*). Our tumor appeared in a DBA mouse that had received repeated paintings with 3-methylcholanthrene. Ordinarily we do not recognize mast cell tumors during autopsies of mice; hence we seldom have opportunity to transplant them, but identify them only on histological examination. If mast cell tumors are suspected, a touch preparation of the tumor tissue, air-dried and stained with toluidine blue, may allow their identification while the tissue is fresh enough to be transplanted. The mast cell tumors of mice consist of rows of tumor cells. They are composed almost entirely of mast cells, in contrast to mast cell tumors of dogs in which mast cells are mixed with other cells such as eosinophils and fibroblasts. We have, indeed, in the mouse mastocytoma, an almost pure mast cell population.

The tumor was continued in transplant by Dr. Michael Potter and has been designated P815 (*3*). It has been transplanted repeatedly and usually grows easily. One feature of our murine mast cell tumor that still puzzles me is the bright-yellow pigmented masses observed in necrotic areas of the tumor. It was the curious color of the original tumor that induced us to transplant it. The pigment persisted throughout the first three or four generations during transplant and then disappeared. I have found no references by other investigators to a similar pigment in mast cell neoplasms, and we have no explanation for it, though this pigmentation resembles the hematoidin pigmentation seen in some reticulum cell neoplasms of mice. Dr. Ragna Rask-Nielsen (*6*) of Denmark has also obtained a mast cell tumor in the mouse, and she did not describe such pigment.

Dr. Potter was able to get our mast cell tumor into an ascites form, which illustrates another curious feature of the tumor. One of the ascites cells has a ring-form nucleus which is characteristic of the granulocyte nucleus in the mouse, and which we would not expect in a mast cell nucleus.

Table 1, provided by Dr. Albert Sjoerdsma, shows the very high content of serotonin of the mouse mast cell tumor, which is not found in other species, and compares the serotonin and histamine contents of normal and pathologic (or neoplastic) mast cells from various sources.

TABLE 1.—Concentrations of serotonin and histamine in mast cells from various sources

Material	Serotonin (μg/g)	Histamine (μg/g)
Tumors		
Mouse mast cell	80–180	470–560
Dog mast cell	<0.2	315, 160
Other mouse tumors	<0.2	<1
Human skin		
Urticaria pigmentosa	<0.25	44
3 normals	<0.7	4.7, 5.4, 5.0

I believe the mouse has much to contribute to our knowledge of mast cells. I hope by now mice are more readily available and additional studies can be undertaken.

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Comparative Aspects of the Mastocytoses[1]

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IN CONTRAST to mast cells of the dog, those of man are not conspicuous in inflammatory or neoplastic diseases. They can be observed in mixtures of inflammatory cells in several chronic diseases of man including ulcerative colitis, interstitial cystitis, chronic cystitis "mastitis," and rheumatoid arthritis (*1*, *2*). They may be present in or near neoplasms, particularly in draining lymph nodes (*2*). They may be mixed with other cells in Hodgkin's disease (*3*). And they are involved in a spectrum of rare entities known as the *mastocytoses.*

Mast cells may be overlooked in histologic sections. Their granules may dissolve in watery fixatives. They may resemble plasma cells (*4–6*), eosinophilic myelocytes, or macrophages. Their nuclei resemble those of lymphocytes; their cytoplasm which is usually abundant stains pink with hematoxylin and eosin and may appear granular, "foamy," or vacuolated.

The *mastocytoses* are related processes of unknown etiologies characterized by non-neoplastic and neoplastic proliferations of mast cells. The most frequent type in man is the benign skin disease *urticaria pigmentosa,* which may progress to a more malignant disseminated form involving internal viscera and may eventually develop all the characteristics of a leukemia, including a fatal termination.

Urticaria pigmentosa is relatively rare; by 1963 (*7*) only 600 cases had been reported. The pathologic lesion is a diffuse accumulation of morphologically "normal" mast cells in the dermis of skin primarily of the trunk and proximal extremities. Histamine released from mast cells causes a characteristic urtication and dermatographism. The brownish pigmentation is an effect of increased numbers of melanocytes that overlie collections of mast cells (*8*). Urticaria pigmentosa usually starts during infancy or early childhood or it may be present at birth. The infantile lesion may be solitary or multiple, tends to be benign, and often disappears by puberty. There are several reports of the disease occurring in more than one member of a family (*2*). One report described a woman who first developed urticaria pigmentosa during pregnancy. The infant born of this pregnancy developed urticaria pigmentosa at 3 months of age; an older sibling, who was born before the onset of the maternal disease, and 2 infants, born of subsequent pregnancies, did not have the disease (*9*). When onset occurs in adults, the process tends to be more severe, and after varying lengths of time (with as many as 30 years intervening) symptoms and signs that mark the beginning of disseminated disease may appear. Caplan (*10*) estimated that one-fifth of patients with multiple lesions of urticaria pigmentosa eventually develop disseminated disease. Disseminated mastocytosis may occur without urticaria pigmentosa. Systemic effects of biochemical substances released from mast cells of the localized skin lesion must be distinguished from effects of proliferations of mast cells in such organs as liver, spleen, lymph nodes, and bone marrow, which may be those of any reticuloendotheliosis or leukemia; this distinction however is not always possible in the clinical situation. Nonspecific changes in bones observed by X ray are complicated, and the direct effects of histamine and heparin on bone have not been convincingly separated from effects of proliferations of mast cells, the accompanying fibrosis, and dietary deficiencies related to the "spruelike" malabsorption syndrome that has been described (*11*). The hyperhistaminemia can explain the tendency to development of peptic ulcers which occurs in both humans (*7*) and animals (*12, 13*) with mastocytoses.

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Even during the disseminated phase, the disease may be entirely benign. Symptoms may be related to effects of biochemical substances released by mast cells or replacement of hematopoietic elements of bone marrow incident to mast cell proliferation and the accompanying fibrosis. In patients with disseminated disease, mast cells infiltrate liver, spleen, lymph nodes, and bone marrow and may circulate in peripheral blood.

The most severe form of systemic mastocytosis is *mast cell leukemia.* The pathologic and hematologic features of systemic mastocytosis and mast cell leukemia have been described by Rappaport (*14*). The type of leukemia present is sometimes difficult to establish, and in blood films and bone marrow, immature mast cells with nonspecific granules are difficult to distinguish from immature granulocytes of other series and monocytes. The granules of mature mast cells are said to be peroxidase-negative, in contrast to those of other granulocytes including blood basophils (*14*). Systemic mastocytosis has reportedly terminated as myelofibrosis-myeloid metaplasia (*2*), monocytic leukemia (*2*), granulocytic leukemia with chloroma (*2*), "acute" leukemia (*2*), Hodgkin's disease (*2, 15*), and lymphoma (*16*). There are two case reports of development of polycythemia vera (*17, 18*). Primary or atypical amyloidosis has also been reported in systemic mast cell disease (*2, 9, 19*) and may be associated with a proteinopathy. Unfortunately an autopsy was not performed on the patient described by Weidman and Franks (*15*), and referred to by Sagher and Evan-Paz (*2*), in whom a pathologic process resembling Hodgkin's disease developed 12 years after onset of urticaria pigmentosa. The lymph nodes of this patient contained mast cells, histiocytes, eosinophils, and giant cells with features of Reed-Sternberg cells. Pleomorphic mast cells tumors of *dogs* may contain multinucleated giant cells (*6*) resembling Reed-Sternberg cells.

In 1963, Mutter and associates (*7*) summarized features of systemic mast cell disease in 29 patients based on findings with one of their own patients and on descriptions of 28 patients in the literature. There were 17 males and 12 females. Six patients were under 8 years of age at onset of the disease, and the remainder were over 30. Of the 29 patients, 24 had urticaria pigmentosa. All 29 patients had clinical evidence of systemic involvement: hepatomegaly and splenomegaly in 27, lymphadenopathy in 10, skeletal abnormalities seen by X ray in 16, and gastrointestinal symptoms in 8. Duodenal ulcers occurred in 4 patients. In 9, levels of urinary 5-hydroxyindoleacetic acid were normal, and in 3, urinary histamine was increased. Anemia of less than 10.0 g/100 ml of blood was recorded in 11 patients, and leukocytosis in excess of 30,000/mm^3 was present in 6 patients. Of these 6 patients, at least 3 probably had mast cell leukemia; mast cells accounted for as many as 50% of circulating cells in peripheral blood. Five additional patients with normal leukocyte counts had mast cells in peripheral blood, 45% of cells in one patient. Mast cells were recorded in bone marrow of 16 patients, of whom 9 also had mast cells in peripheral blood; mast cells are rarely observed in normal bone marrow. Interestingly, Asboe-Hansen (*20*) described a patient who had up to 14% blood basophilic granulocytes in peripheral blood and "many" mast cells in bone marrow; unfortunately, the author did not characterize the basophilic leukocytes and did not include photographs of cells. Rebuck *et al.* (*1*) presented evidence of a definite but incompletely understood relationship between blood basophilic granulocytes and tissue mast cells. Although mature forms of the two cells are morphologically distinct, basophils and mast cells are similar in the basophilic granulation and content of similar biochemical substances. Cells of both types may be present in exudates of experimentally produced "skin windows" and in spontaneous inflammatory lesions of patients with ulcerative colitis and interstitial cystitis. Nielsen (*12*) commented on the many reports of basophilic leukemia in cats with systemic mast cell disease and emphasized the difficulties in differentiating mast cells from basophilic granulocytes. The fact that cells of the hematopoietic system arise from identical precursors suggests an explanation for the occasional participation of mast cells in pan-myeloproliferative processes.

Sagher and Evan-Paz summarized features of 71 "proved" and 74 "possible" cases of patients with systemic mastocytosis. This summary, based on the authors' own experience and on reports in the world literature, included observations made at necropsy of 22 patients. The descriptions of the "proved" cases convincingly show that disseminated disease was present. Only 26 of the 71 "proved" reported cases were fatal within the time of the study (1949–65), indicating that a chronic and relatively benign course is characteristic for most patients with the disseminated form of the disease. Most fatal cases terminated as a leukemic process. The course of disease and terminal events were those of any leukemia. Cases recorded as myelofibrosis and/or myeloid metaplasia were difficult to interpret, because fibrosis of bone marrow is frequent in mastocytosis and the resulting extramedullary hematopoiesis may be confused with leukemia. The bone lesions in mastocytoses have not been completely characterized pathologically and have been described both as osteoporotic and osteosclerotic. Fibrosis of marrow cavities and increase in bone formation with encroachment of marrow cavities have been described with and without demonstration of mast cells in the fibrotic areas. To explain the many puzzling phenomena observed in the mastocytoses, more complete information is needed on the precise functions of mast cells in physiologic and pathologic conditions. Mast cells are particularly notorious for their associations with collagen and paradoxically, or so it seems, appear to be involved in both constructive and destructive processes of collagen and bone.

The interrelations of mast cells with other cells of the immune reactive system are poorly understood. The roles of mast cells in diseases of altered immune reactivity are discussed by Nixon (*16*) and by King (*21*). Nixon observed Waldenström's macroglobulinemia in a patient with urticaria pigmentosa, who had increased numbers of mast cells in bone marrow and cited two similar cases in the literature (*16*). The involvement of mast cells in Waldenström's macroglobulinemia is illustrated by reports of Tischendorf and Hartmann (*22*) and by Waldenström (*23*), who observed increased numbers of mast cells mixed with lymphocytes and plasmacytoid cells in bone marrow of patients with this syndrome. Nixon (*16*) also described a lymphoma in a patient with urticaria pigmentosa. Carr and Mengel (*24*) reported that a 41-year-old man with systemic mastocytosis developed a peculiar lesion of trunk and lower extremities which resembled Kaposi's sarcoma.

Most recent reports have indicated that serum proteins of patients with systemic mastocytosis are usually unremarkable, as indicated by biochemical methods and electrophoresis (*8, 25, 26*), although abnormal serum globulins have been reported in dogs with mast cell neoplasms (*27*).

Five cases of systemic mast cell disease in humans have been recorded since Sagher and Evan-Paz's review (*8, 25, 26, 28, 29*). Fitzpatrick (*8*) saw 8 patients with systemic mastocytosis in the Boston area during a 4-year period which indicates that the disease is probably being recognized more frequently.

Despite the powerful pharmacologic potential of biochemical substances released from and presumably synthesized by mast cells, there is little information concerning effects of substances extracted from human mast cells, except for histamine. The elevated blood histamine levels account for the prominent urtication and probably the frequent peptic ulcers observed in man (*7*), dogs (*13*), and cats (*12*) with mast cell diseases. There have been no convincing reports of alterations in coagulability of blood associated with excess of heparin in human patients, although slight increases in clotting times, measured by a capillary tube technique, were reported in 5 of 14 dogs with mast cell tumors (*30*). The explanations of the hemorrhages observed in some patients with mast cell diseases are complicated by the fact that thrombocytopenia sometimes occurs despite normal numbers of megakaryocytes in bone marrow. Five patients in the review of Mutter *et al.* had platelet counts less than 50,000/mm^3 (*7*).

Therapy has been primarily aimed at relief of symptoms caused by histamine. There have been too few documented cases of systemic mastocytoses to assess therapy aimed at destruction of cells.

Serotonin has not been demonstrated in human mast cells. Most attempts to demonstrate increased urinary 5-hydroxyindoleacetic acid have been unsuccessful, with the possible exception of the case of a 3-year-old girl with urticaria pigmentosa in whom a "tripling" of the level occurred following administration of codeine (*31*); this report, however, is difficult to interpret from evidence in the paper.

Cutaneous mast cell tumors are among the most frequent mesenchymal skin neoplasms of dogs. They are ordinarily benign but may recur following surgical removal, may invade deep structures locally, and may metastasize. Mast cells can be seen in draining lymph nodes and viscera of many dogs with mastocytomas if a careful search is made (*6*, *13*). The pleomorphic mast cell tumors composed of immature or atypical mast cells are occasionally difficult to distinguish from lymphomas (*6*). Such tumors may contain polymorphonuclear eosinophilic leukocytes, fibrosis, and multinucleated giant cells and may resemble human Hodgkin's disease (*6*).

Mastocytoses of man and dogs are similar in that potentially malignant processes originate in skin. The initial canine skin disease is a neoplasm; the human urticaria pigmentosa is a diffuse non-neoplastic infiltration of mature mast cells. The cells of the canine mast cell tumor may be well differentiated, anaplastic, or intermediate, and the tendency to disseminate correlates with the degree of anaplasia (*13*). Although human urticaria pigmentosa may progress to a disseminated form, local invasion by mast cells of structures underlying involved skin has not been described. However, the disseminated phase of disease in man and dogs is similar, and both species can develop mast cell leukemia. The rare visceral mastocytoses of cats and cattle are also similar to disseminated mast cell disease of man.

Mast cell neoplasms are rare in cats; fewer than 50 cases have been reported. In cats the disease may manifest as localized skin lesions but has been observed more frequently as a disseminated disease (*12*).

HEMATOPOIETIC NEOPLASMS

Mastocytomas are rare in cattle, and generalized processes involving internal viscera and blood have been reported (*32*, *33*). Mast cell tumors have been reported only in skin of pigs (*33*). Both spontaneous and chemically induced mast cell neoplasms occur in mice (*34*).

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Burkitt's Lymphoma—A Comparative Study[1,2]

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SUMMARY

The Burkitt lymphoma is a form of human malignant lymphoma which shares certain clinical and histologic features with lymphomas of animals. The visceral lymphosarcoma of domestic cats appears to resemble the Burkitt tumor in its clinical and pathologic features and possibly its response to chemotherapy.—Nat Cancer Inst Monogr 32: 297–301, 1969.

BURKITT'S LYMPHOMA is a tumor syndrome originally described in African children, but observed elsewhere in the world. It is characterized by nonleukemic lymphoid tumors of extranodal sites as bones, particularly jaws, and viscera, particularly ovaries (*1–4*). Tumor cells are undifferentiated lymphoid cells which may contain intracytoplasmic lipid. Active macrophages create the familiar "starry sky" appearance. The tumors appear to be uniquely sensitive to cytotoxic drugs (*5*). No single feature is pathognomonic of the syndrome (*6*). Rather, the total disease presentation including anatomic, clinical, histologic, and cytologic features must be considered in making a diagnosis of a Burkitt tumor.

Like Hodgkin's disease, Burkitt's lymphoma is defined as a human disease and, unless a common etiologic agent or process is identified in animals and man, we can only point out similarities between disease counterparts. Of the common animal lymphomas, the visceral lymphosarcoma of domestic cats most nearly resembles the Burkitt tumor (*7*). Similar neoplasms have also been described in dogs (*8, 9*), in a primate (*10*), and in other animals (*11*). Comparisons to Burkitt's lymphomas in these reports were based on histologic appearances, with emphasis on presence of "starry

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sky" phenomena. If histologic similarities are the only bases for diagnoses of Burkitt's tumors, it is likely that lymphomas occurring in most species could qualify.

FELINE VISCERAL LYMPHOSARCOMA

The visceral lymphosarcoma, the common form of lymphoma of cats, exhibits several striking anatomic and clinical similarities to the Burkitt lymphoma. This tumor is the most common malignant neoplasm of domestic cats in the United States (*12*) and recent reports implicate a probable viral etiology (*13*, *14*). Epidemiologic evidence suggests that horizontal transmission of infectious agents may occur (*15*). Although feline lymphomas may manifest as a disseminated disease similar to lymphomas of dogs, involvement is more frequently limited to visceral organs without infiltrations of peripheral lymph nodes and without leukemia (*16*).

The predominant organs affected are kidneys, small intestine and mesenteric nodes, anterior mediastinum, liver, and spleen. Of 17 cats studied in our laboratory, 2 had involvement of the mandible and 1 had a primary lymphoma of the ovary. No other tumors were found in the latter animal, although paraplegia was observed clinically. Because peripheral adenopathy and leukemia are rarely seen in lymphomas of cats, diagnoses may be difficult unless the examiner is familiar with the visceral lymphosarcoma syndrome. The clinical course in untreated cats is ordinarily short; several animals in our series were observed to be in terminal stages of disease less than 2 weeks after primary signs were first noted.

The predominant cells of the feline tumors are large, primitive lymphoid cells which, by our cytologic criteria, are prolymphocytes (*17*). Lymphomas of approximately half the cats in our series contained numerous active macrophages scattered among the tumor cells producing the "starry sky" pattern (fig. 1). In imprints, cytoplasm of macrophages was broad and coarsely vacuolated and contained cellular debris and other particles which stained with periodic acid-Schiff, oil red O, and methyl green-pyronine. Many lymphocytic cells also contained lipid droplets, a feature characteristic of cells of the Burkitt tumor.

Fresh imprints of tumors were studied by histochemical methods for acid phosphatase, nonspecific esterase, and β-galactosidase. The enzyme reactivities were very strong in active macrophages, as well as in resting histiocytes.

We have treated visceral lymphosarcomas of 4 cats by chemotherapy. Two cats had primary renal lymphosarcoma, one had a primary intestinal tumor, and the last one had jaw involvement. In each, marked tumor regression was achieved 7–10 days after a single intravenous injection of 15 mg/kg of cyclophosphamide. The cats were killed 14–21 days after therapy because our facilities did not permit prolonged medical care.

There was no gross or microscopic evidence of residual tumor in any of the animals (figs. 2 and 3). Additional studies of effects of chemotherapeutic agents on feline lymphomas are in progress to determine whether the apparent sensitivity of these tumors is a consistent feature.

DISCUSSION

Similarities can be found between lymphomas of animals of most species as they can between hepatomas or other tumors. Most lymphomas of domestic animals differ from the human Burkitt tumor in several respects: There is often widespread involvement of lymph nodes, leukemia is frequent, and drug sensitivity is variable. Feline lymphomas, however, show many features characteristic of Burkitt's lymphoma. The predominant tissues involved in the feline disease are visceral organs, usually without generalized lymphadenopathy or leukemia.

The tumors consist of immature lymphoid cells which may contain lipid droplets and active phagocytic histiocytes often associated with "starry skies."

Preliminary evidence indicates that some feline lymphomas may resemble Burkitt's lymphoma in their sensitivity to chemotherapeutic agents. One explanation of the remarkable chemosensitivity of Burkitt's lymphoma in children might be that the response to some degree depends on the immune capacity of the host (*18*) and may be related to previous or concomitant stimulation of the reticuloendothelial system in response to infection by malaria or other parasites (*19*). Domestic cats are reported to have a high incidence of infection by a protozoan parasite of erythrocytes, *Hemobartonella felis* (*20*); it is tempting to speculate that this or other parasites may have an effect on the feline lymphoid tumors.

The prominent phagocytosis in Burkitt tumors, other types of lymphomas of man and animals, and in active non-neoplastic lymphoid tissues may reflect rates of cell death. We have noted development of a "starry sky" phenomenon in lymphomas of dogs during drug-induced remissions. The degree of phagocytic or lysosomal activity, as revealed in semiquantitative enzyme histochemical preparations of canine lymphomas, correlates well with the sensitivity of tumors to drugs. Similar studies of AK mouse lymphomas, which are extremely sensitive to both prednisone and cyclophosphamide, have revealed very strong acid-phosphatase activity in numerous macrophages and in many tumor cells. The "starry sky" appearance in the Burkitt and other lymphomas may, therefore, be a visible manifestation of a host defense active against the tumor.

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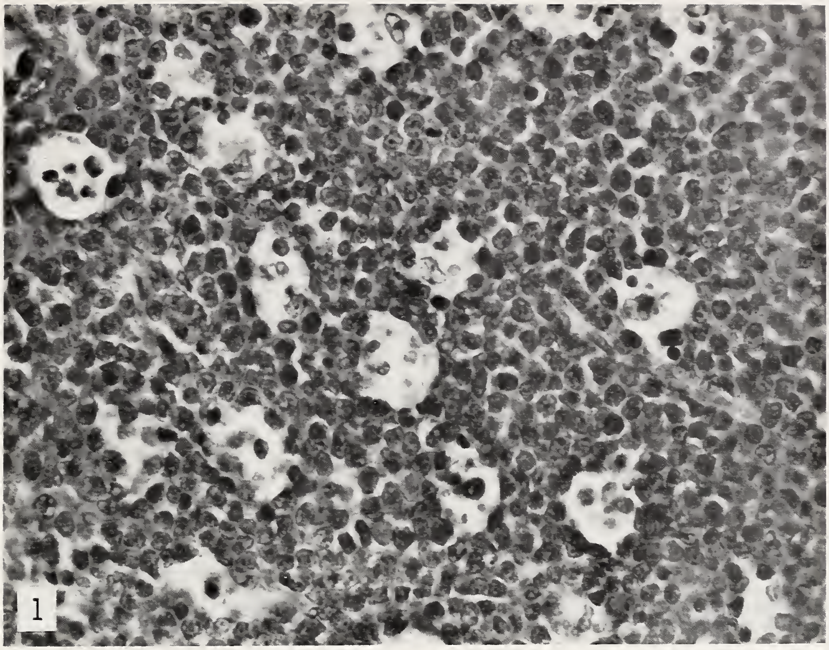

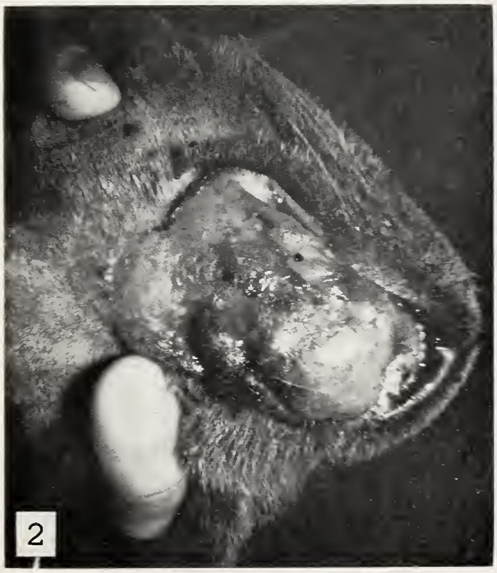

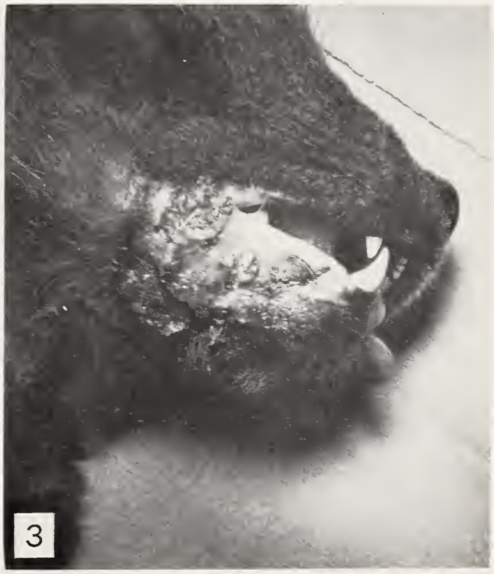

FIGURE 1.—Section of feline visceral lymphosarcoma showing prominent "starry sky" effect. Hematoxylin and eosin. × 500

FIGURE 2.—Lymphosarcoma of mandible and surrounding soft tissues in a cat.

FIGURE 3.—Same animal as shown in figure 2, one week after a single intravenous administration of 15 mg/kg cyclophosphamide.

Plasma Cell Neoplasms of Man and Animals[1]

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PLASMA CELL NEOPLASMS OF MAN

NEOPLASTIC DISEASES of plasma cells and other immunocytes are a complex of entities that share the common feature of anomalous serum globulin formation and are known as *proteinopathies*. Most proteinopathies are destructive and rapidly lethal neoplasms; others follow a prolonged course, and evidence of the immunoglobulin anomaly may antedate development of a frank neoplasm by months or years. Most frequently, neoplastic cells are recognizable as plasma cells or their immediate precursors; in some patients neoplastic cells are lymphocytes, intermediate forms between lymphocytes and plasma cells, undifferentiated cells, or reticulum cells. Since the morphologic characteristics of plasma cell neoplasms and lymphomas overlap, the distinction is not always clear.

The most frequent form of plasma cell neoplasm of man is the clinicopathologic syndrome, *plasma cell myeloma* or *multiple myeloma* (*1*). "Myeloma cells," which vary from undifferentiated or reticulum cells to mature plasma cells, characteristically infiltrate and replace bone marrow, destroy bone, and produce increased amounts of serum globulins of a single immunoglobulin class (*2–4*). Bone destruction is associated with pain, pathologic fractures, and radiologic osteolytic lesions. Patients with the myeloma syndrome are unusually susceptible to bacterial infections and exhibit defects in formation of serum antibodies to bacterial antigens. Despite extensive involvement of bone marrow by myeloma cells, plasma cell leukemia is rare.

Approximately 90% of the patients with disseminated plasma cell myelomatosis produce homogeneous serum globulins called myeloma proteins, M-components, or paraproteins. These globulins are indistinguishable from normal immunoglobulins by biochemical and immunologic techniques and may be IgG, IgA, IgM, IgD, or IgE (IgND) types (*2–4*). Sixty-one percent of the patients of one series produced globulins of the IgG type (*5*). IgM, or macroglobulins, are more characteristic of Waldenström's macroglobulinemia than of myelomas (*1*). Myeloma globulins occasionally exhibit cold precipitability (cryoglobulins) or, rarely, may be pyroglobulins. The globulin produced by an individual retains its class type throughout the disease. A few patients produce more than one type of globulin. The single, narrow serum electrophoretic band, or "spike," produced by neoplastic immunocytes is different from the broad, diffuse bands of immune stimulation. Myeloma proteins do not show specific antibody activity; if they are produced in response to antigenic stimulation, the antigens are not known. More likely, production of myeloma proteins is a nonspecific expression of functional differentiation of neoplastic immunocytes. Immunoglobulins and their counterparts in neoplastic diseases have been described by Fahey and other investigators whose publications should be consulted for more complete information (*2–4*).

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[1] This paper summarizes information on this topic presented in formal papers and informal discussions during the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Total serum proteins may be normal or elevated in myelomatosis; levels range from 5 to 15.3 g/100 ml, with an average of 9.0/100 ml (*6*). Elevated serum globulins cause pseudoagglutination of erythrocytes (rouleaux); focal anoxias and hemorrhages are two manifestations of the "hyperviscosity" syndrome of patients with these disorders.

Bence Jones proteins are light chain subunits of immunoglobulins with molecular weights in the range of 22,000 produced in 50–60% of the patients with the myeloma syndrome. The peculiar thermal coagulability of the urinary Bence Jones proteins, which solidify at 45–55°C and redissolve at higher temperatures, was discovered in 1845, not by Henry Bence Jones, who described many of the interesting characteristics of the proteins, but by an English physician, William MacIntyre (*7*), whose patient had the first recorded case of myeloma. Electrophoretic analysis of concentrated urinary proteins was subsequently shown to be a more accurate method of identification of Bence Jones proteins than the heating procedure.

Neither irradiation nor drugs have produced sustained remissions (*5*), and survival of patients with disseminated myelomas is usually less than 2 years following diagnosis, although a few patients survive 5 or more years. Survival cannot be correlated with production of abnormal proteins or their type; some investigators believe that individuals with better differentiated cell types live longer than those with relatively undifferentiated types.

Necropsies of patients with myelomatosis reveal widespread infiltrates of neoplastic plasmacytes in bone and extramedullary sites including lymph nodes and internal viscera. Approximately 10% of patients show atypical amyloid deposits in bone, muscle, or other structures. Many patients who present as primary amyloidosis ultimately are found to have myeloma. Clinical uremia may develop in patients with myeloma; kidney tubules of these patients are often filled with crystalloid proteinaceous casts, some of which are surrounded by foreign-body giant cells and polymorphonuclear leukocytes, and the tubular epithelium may show degeneration (*8*). These renal changes, the so-called "myeloma kidneys," are not limited to patients who excrete urinary Bence Jones proteins.

Plasmacytomas may occur in various extramedullary sites, such as the upper respiratory tract, the gastrointestinal tract, the spleen, lymph nodes, or other sites (*9–13*). They occur most frequently in structures of the oronasopharynx. Extramedullary plasmacytomas are less frequent than those arising in bone marrow, although precise statistics for each type are not available and few tumor registries have accumulated enough cases to provide accurate assessments. Many features suggest that extramedullary plasmacytomas are more similar to lymphomas than to myelomas. Like lymphomas, extramedullary plasmacytomas tend to remain localized in primary sites for varying periods of time and are potentially curable by surgery or irradiation. After removal they may recur or, after varying periods of time, may disseminate to involve other structures, including bone and bone marrow. The disseminated phase may occur years after the localized primary tumor first appears. When involvement of bone and other structures is widespread, the disease cannot be distinguished from primary myeloma. During localized stages, extramedullary plasmacytomas may or may not produce globulins; during disseminated phases myeloma proteins are usually produced. Despite evidence that plasma cell formation is prominent in lymph nodes during immune stimulation (*14*), fewer than 10 cases of primary plasmacytomas of lymph nodes have been reported in the literature from the United States (*12*). Plasma cell neoplasms are sometimes difficult to distinguish from inflammatory reactions to a variety of stimuli.

Less frequent syndromes involving anomalous protein formation are Waldenström's macroglobulinemia (*1, 15, 16*) and heavy chain disease (*1*). The pathologic lesions associated with the rarer proteinopathies vary and may be non-neoplastic proliferations of various immunocytes or frank neoplasms composed of plasmacytes, or lymphomas.

Epidemiology

Plasma cell neoplasms account for 11% of all fatal hematopoietic neoplasms of man, in whom they cause death at an annual rate of 1.7/100,000 population (*17, 18*). More than 95% are disseminated or multiple myelomas. The remainder are a variety of proteinopathic syndromes for which precise incidences are not known. Myeloma is a disease of adults past 30, although it occasionally affects individuals in the second decade. Incidence increases with age. A report of a myeloma in a 7-year-old child did not include convincing evidence that a neoplasm was present, and a necropsy was not performed (*19*).

Statistics from the United States have consistently indicated higher rates of multiple myeloma in Negro males than in Caucasian males (*20, 21*). In Brooklyn, New York, myeloma occurred in Negro males at twice the rate for Caucasian males (*21*). In 1964 (*17*) plasma cell myelomas caused deaths in U.S. males designated as "nonwhite" at a rate of 2.3/100,000. The rate for "white" males was 1.8 and rates for white and nonwhite females were 1.5 and 1.6; the average for all groups was 1.7/100,000. The higher rates of myelomas recorded for "nonwhite" males contrast sharply with those for all other hematopoietic neoplasms, which are distinctly less frequent in "nonwhite" individuals of all ages (*17*). Myeloma occurs in Negro males nearly a decade earlier than in Caucasians (*21*).

Information of incidence of plasma cell neoplasms in countries other than the United States is difficult to obtain because of the unfortunate tendency to lump statistics for myelomas with those of lymphomas or other hematopoietic neoplasms (*22*). A World Health Organization report (*23*) provided myeloma rates for 16 selected countries for the years 1949–53. Rates were recorded for Canada, the United States, Japan, Germany, Denmark, Finland, Ireland, Italy, Norway, Netherlands, the United Kingdom, Scotland, Sweden, Switzerland, Australia, and New Zealand, although complete information was not available for all countries. Annual crude rates for myelomas averaged 1.0/100,000 in the United States and Canada for the time period studied, lower than the 1964 rates for the United States (*17*). The lowest rates of all countries were recorded in Finland and Australia (0.3 and 0.6/100,000, respectively). Worldwide, the peak age was 60–70 years, except for nonwhite Americans in whom the peak occurred 10–20 years earlier. The peak age was also slightly lower in Italy and Finland than in other countries.

Because of the higher rates of myelomas in American Negroes, a special search was made for information concerning incidences among Negro people elsewhere in the world.

Oettlé reported relative frequencies of plasma cell neoplasms in 50,000 cancers observed in the South African Institute for Medical Research, Johannesburg, South Africa, for the years 1949–53; this study involved a population of approximately 1,200,000 "white" and almost 3,500,000 Bantus (*24*). Myeloma appeared to be the only lymphoreticular neoplasm that was at least as common in Bantus as in whites, although only a few cases were studied. The rates for white and Bantu males were nearly equal (0.30 and 0.31/100,000, respectively); the peak age was approximately a decade earlier in Bantus. For all other lymphoreticular neoplasms, the rates for Bantus were one-fourth to one-half those for whites. Oettlé stated that, because accurate population figures and health statistics were not available for Bantus of South Africa, reliable incidence figures could not be determined (*24, 25*).

Davies recorded only 15 plasma cell tumors (2 localized plasmacytomas and 13 myelomas) of a total of 822 hematopoietic neoplasms histologically confirmed by the Government Medical Laboratory and Kampala Cancer Survey in Uganda (*26*). Of these patients, 282 were under 15 years; the remainder were adults.

Schonland and Bradshaw (*27*) compared age-adjusted incidence rates of lymphoreticular malignancies in four subpopulations of Durban, Natal, South Africa. Myelomas occurred at higher rates in "African" females (2.8/100,000) and males (1.9) than in Indians (0.5 and 0.7), Moslems (0 and 0), or Hindus (0.5 and 0.8) living in the area.

McFarlane (*28*) estimated the annual rate of multiple myeloma in West Indian Negroes of Jamaica to be in the range of 5/100,000, a rate considerably higher than recorded elsewhere in the world.

Familial multiple myeloma has been recorded (*29*); the disease occurred in a 58-year-old man, 1 of his 4 daughters at age 66, and 1 of 2 sons at age 67.

PLASMA CELL NEOPLASMS OF ANIMALS

Spontaneous plasmacytic neoplasms appear to be relatively infrequent in animals compared to man, although this impression should be reevaluated after additional information is accumulated.

Plasma cell neoplasms have been reported in dogs (*30–40*), a horse (*41*), a cat (*42*), mice (*43–55*), hamsters (*56, 57*), a rat (*58*), and mastomys (*59*). Squire (*60*) cited additional reports of plasma cell neoplasms in dogs, a calf, and a pig. They have been observed in a gibbon (*61*) and in 2 African green monkeys (*62*).

Descriptions of the canine disease indicate many similarities to plasma cell myelomas of man (*30–40*). All dogs had proliferations of plasmacytes in bone marrow and in other sites. Bone lesions were demonstrated by X ray in 6 dogs, urinary globulins (Bence Jones) in 3, and abnormal serum globulins in 4 (*36, 37, 39, 40*).

Spontaneous plasmacytic neoplasms of C3H mice were reported to arise most frequently in the region of the ileocecal valve of old mice (*44*). These mice did not have urinary Bence Jones proteins, but some had abnormal serum proteins.

The "plasma cell leukemias" of BALB/c mice occurring after exposure to "subcellular agents" from mice of a different strain, reported by Ebbesen, Rask-Nielsen, and McIntire (*51*), were diffuse neoplasms involving lymphoreticular structures, and neoplastic cells ranged from undifferentiated reticular cells to mature plasma cells. Plasma cells were only occasionally observed in peripheral blood. One-third of these mice had elevation of a single serum immunoglobulin. Many male mice developed amyloidosis. Plasma cell neoplasms have also been described in DBA, C57BL, CBA, and AK mice and in various hybrids (*45*). They can be produced in BALB/c mice by intraperitoneal injections of mineral oil; Potter and MacCardle (*48*) traced the pathogenesis of the neoplasm from a granuloma of the mesentery. Similar neoplasms can also be induced by intraperitoneal inoculation of plastics such as lucite shavings (*52*).

Potter and Fahey (*46*) reported that serum proteins of mice with transplantable plasma cell neoplasms were distinct and characteristic for each neoplasm, and that the capacity to produce the same proteins was maintained during transplantation. Plasma cells and paraproteins are conspicuous components of the pleomorphic reticular neoplasms of SJL/J mice (*55*).

Of animal plasma cell tumors described to date, those of the dog are most comparable to the "multiple" myeloma syndrome of man, since they originate in bone marrow, cause destructive bone lesions, and produce paraproteins.

Aleutian disease (*63*), an infectious disease of mink believed to be transmitted by a virus and characterized by widespread diffuse infiltrations of plasma cells and increased serum globulins, while not a neoplasm, is a useful laboratory model for investigations of immunoglobulin synthesis.

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Protein Production. A Possible Unifying Concept of Reticuloendothelial Disease[1]

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THE FOLLOWING observations suggest possible interrelations between plasmacytomas, Hodgkin's-like diseases, and mast cell diseases.

Human disorders involving immune globulins can at times show a lymphohistiocytic pattern resembling Hodgkin's disease. Occasionally late in the disease, patients with plasmacytomas (multiple myelomas) develop a histologic pattern difficult to distinguish from Hodgkin's disease or pleomorphic reticulum cell sarcoma. These changes may be effects of therapy. However, these patients continue to produce large amounts of protein with characteristic spikes in the globulin range. Cells composing these neoplasms can include those that are morphologically indistinguishable from Reed-Sternberg cells. Studies of serum proteins in patients with atypical reticular neoplasms may assist in elucidating the true nature of these diseases. Another point to be made in this discussion is that extramedullary plasmacytomas in multiple myeloma can present as a macroglobulinemia (*1*). In addition, mast cells are frequently associated with human disorders of immune globulins, particularly macroglobulinemia. These observations suggest that investigations of immune globulins in animals with plasma cell lesions, mast cell lesions, and Hodgkin's-like lesions may provide important information, although the processes in animals may not be identical to those seen in man.

In my investigation of a canine plasmacytoma, no increase in IgM, IgA, or IgG could be demonstrated by immunologic methods in which human antisera were used. However, an apparent increase in globulins was demonstrated by electrophoresis when human standards were used. These findings support observations of other investigators who suggest that each animal has its own species-specific antigenic serum proteins but that these may have human counterparts by other criteria (*2*).

Three reports of canine myelomas include investigations of serum immunoglobulins. A canine gamma A myeloma was reported by Rockey and Schwartzman (*3*). A canine myeloma reported by von Niepage *et al.* (*4*) was associated with an increase in serum globulin that had polymeric features similar to those found in gamma A myeloma of man. The myeloma reported by Medway *et al.* (*5*) appears to be one of the most thoroughly studied canine myelomas in the veterinary literature. Investigations of canine immunoglobulins are now in progress in many centers (*2*, *6*, *7*), but no authoritative list of normal serum protein values for dogs is available for comparison. Studies of both normal and abnormal globulins of dogs and other animals are needed to provide baselines for such comparisons. One of the better methods for investigating immunoglobulin problems is the procedure used by Welton *et al.* (*1*).

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[2] The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

The neoplasms discussed here are those of tissue that acts as an immunologic defense mechanism under normal conditions; it would be desirable to determine to what degree the abnormal immunologic patterns associated with these tumors can be correlated with histologic patterns.

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Transmissible Venereal Tumors of Dogs[1]

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SUMMARY

The transmissible venereal tumor occurs primarily on the external genitalia of sexually mature male and female dogs. It is transmitted by coitus, persists for long periods, and frequently regresses spontaneously. Not all affected dogs recover, and metastases occur but are not common. Circulating antibodies against tumor cells have been demonstrated. Recent work suggests a viral etiology. Cytogenetic studies of tumor cells revealed a constant count of 59 chromosomes, compared to the 78 chromosomes found in cells of the normal dog. Gross and microscopic characteristics of this neoplasm are described.—Nat Cancer Inst Monogr 32: 315–320, 1969.

ALMOST a century and a half ago the transmissible venereal tumor (TVT) was first described. Since then, it has proved one of the most confusing but intriguing neoplasms affecting dogs. Although Novinsky (*1*) was not the first to describe the TVT, he is considered to be the pioneer in the initial studies of transmission.

Through the years, this tumor has been known as Sticker tumor, venereal granuloma, transmissible sarcoma, transmissible lymphosarcoma, histiocytoma, and several other names, but for this discussion, the term "transmissible venereal tumor" will be used.

The geographic distribution of the TVT is worldwide, although it appears to be more prevalent in the temperate zones. TVT is enzootic in many areas of the world, such as the southeastern United States, Puerto Rico, and Japan. Countries in cooler climates, such as Sweden, are relatively free of the tumor (*2*).

The dog is the only natural host for the TVT. The TVT is transmitted from dog to dog by coitus and possibly by other social behavioral characteristics peculiar to dogs. It is found on or near the external genitalia of sexually mature dogs. In the male, it appears most commonly in the penis, sheath, and scrotum, whereas, in the female, it appears most commonly in the vagina and vulva. Extragenital TVT's have been reported, but to my knowledge, experimental transmission of extragenital tumors has not been successful.

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Progression or growth of a TVT in an affected dog is generally slow, which favors perpetuation and provides opportunities for transmitting the neoplasm. Until quite recently, transmission of this tumor from an infected animal to a susceptible one was considered possible only by transplantation of viable tumor cells—in effect, transplant of a whole cell. This concept was supported by the fact that, in affected dogs of both sexes, a large number of tumor cells, together with a heavy infiltrate of leukocytes and erythrocytes, exist in a serous fluid exuded from the surface of the tumor. Coitus in the dog is prolonged and traumatic; slight injuries to mucous membranes apparently facilitate the transmission of whole cells. Karlson and Mann (*3*) transferred a TVT by whole-cell transplants through 40 successive generations of dogs over a period of 7 years. Other authors have reported difficulty in transmitting this tumor by other than viable tumor cells.

In 1962, and again in 1964, two Italian investigators (*4*, *5*) successfully transmitted a TVT by cell-free materials. McKenna and Prier (*6*) reported that infected dogs develop circulating antibodies to TVT, and they considered this to be evidence of a possible viral etiology. Investigators have known for some time that many affected dogs recover spontaneously from TVT and thereafter are refractory to reinfection. However, not all dogs recover from TVT. Metastatic tumors are not uncommon (*7*). But early surgical excision of TVT usually results in cure, and recurrence is rare.

The gross appearance of a TVT depends primarily on its location. Commonly an elevated tumor, TVT has a polypoid, pedunculated, papillary, or nodular structure. One or more primary tumors may be present. Often multilobulated, TVT is firm and reddish or reddish gray. Frequently, the surface is ulcerated and inflamed (*8–10*) (fig. 1).

TVT is difficult to distinguish microscopically from other neoplasms, especially if the clinical history is vague and the anatomic location is in doubt. Histiocytoma, the mast cell tumor, and malignant lymphoma must be considered in differential diagnosis. The microscopic structure of a TVT is that of a raised tumor composed of solid sheets of cells and interlaced by a delicate fibrous stroma (fig. 2). Occasionally the stroma may divide the cells into clusters or cords simulating an endocrine pattern. The blood supply is usually adequate, although areas of necrosis may be present. Individual tumor cells are uniform in size, spherical to slightly oval, and resemble large lymphoblasts or histiocytes. The shapes of individual cells are affected by regional pressure and trauma. Nuclei are large, vesicular, or hyperchromatic and contain a single, prominent, eccentrically located nucleolus. The pale eosinophilic cytoplasm is scant.

Cell borders may be distinct or indistinct, depending on the degree of crowding (fig. 3). Mitotic activity is very high; as many as 8 mitotic figures may be seen per high-power field. Inflammatory cell infiltrates, predominantly lymphocytes or eosinophils, are usually observed. A photomicrograph of a cutaneous histiocytoma illustrates the problem in differentiation (fig. 4). Without a clear clinical history, an accurate diagnosis would be difficult to make. The main features distinguishing a TVT from a histiocytoma are the differences in the size and shape of their nuclei and the generally paler nuclei and absence of prominent nucleoli in most cells of the histiocytoma. In differentiating the mast cell tumor, a Giemsa or a similar stain should be used in an attempt to demonstrate metachromatic granules. Such a stain brings out the prominent nucleoli of TVT cells more clearly than hematoxylin and eosin.

Interesting recent research on the TVT is concerned with the number of chromosomes in tumor cells. The normal dog has 78 chromosomes, all of which are acrocentric except the sex pair, which are metacentric. Initial work done by Makino (*11*), and confirmed by Weber *et al.* (*12*), indicates that cells of the TVT contain 59 chromosomes. Makino also noted that these cells had 17 metacentric and 42 acrocentric chromosomes. Both investigators observed similar stemlines. The TVT's found in Japan (Makino) and Pennsylvania (Weber) have nearly identical karyotypes. TVT-cell karyotypes do not resemble normal chromosomes of the dog.

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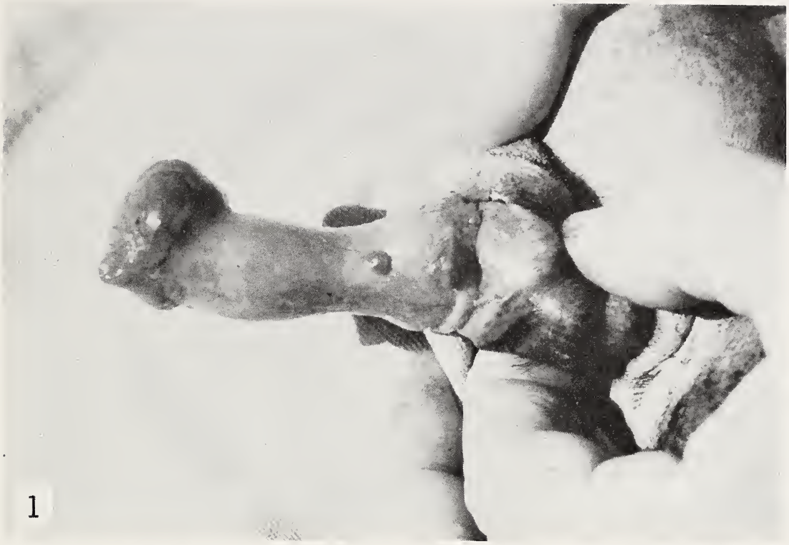


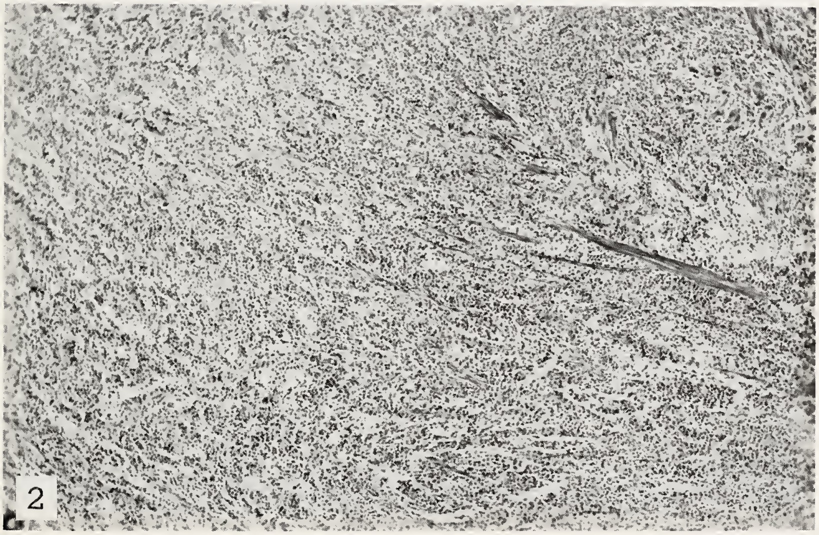
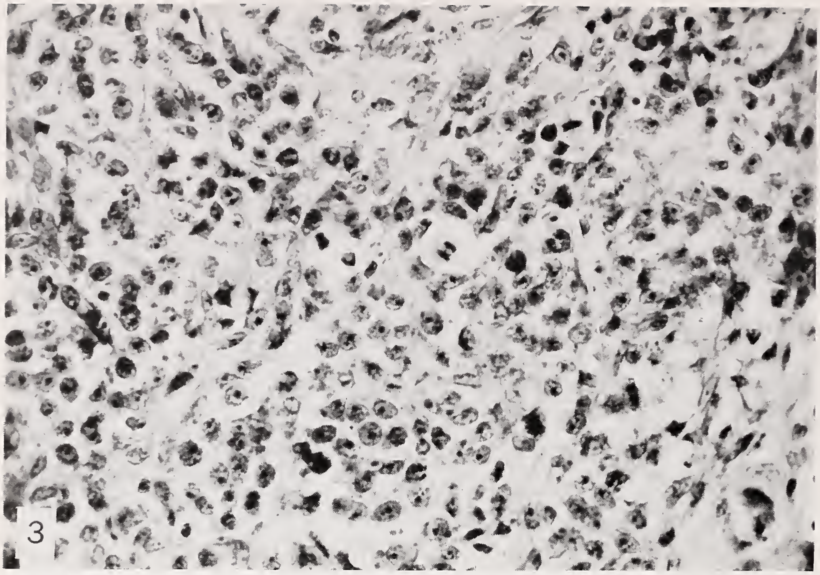


FIGURE 1.—Gross photograph of multiple transmissible venereal tumors on penis of dog. AFIP negative 53-7506.

FIGURE 2.—Photomicrograph of transmissible venereal tumor. AFIP negative 59-6199. Hematoxylin and eosin. × 35



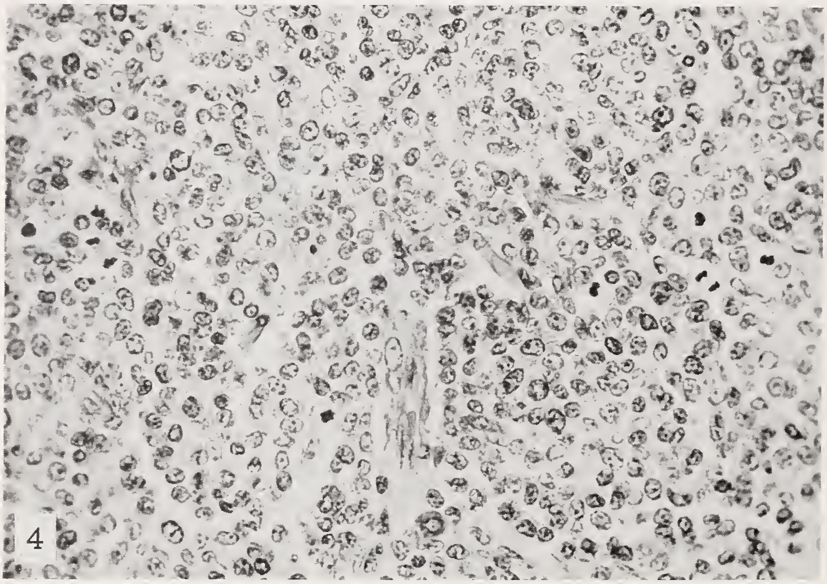


Figure 3.—Higher magnification of transmissible venereal tumor. Prominent, eccentrically located nucleoli and high mitotic activity are the most notable features. AFIP negative 57-8733. Hematoxylin and eosin. × 400

Figure 4.—Canine histiocytoma. Similarity to transmissible venereal tumor (fig. 3) is striking and illustrates the difficulty in differential diagnosis. General cell characteristics and mitotic activity are similar, but prominent nucleoli are absent. AFIP negative 68-3295. Hematoxylin and eosin. × 350

Cutaneous Histiocytomas of Dogs [1, 2]

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SUMMARY

A study of 376 cutaneous histiocytomas supports evidence from other reports that this benign dermal tumor occurs predominantly in young dogs and that more than 45% occur in skin of head and forelegs. Dogs of the boxer breed appear to be unusually susceptible. The tumor must be distinguished from the mastocytoma, the transmissible venereal tumor, and the rare cutaneous lymphomas. There is no known counterpart in other animals or in man.—Nat Cancer Inst Monogr 32: 321–327, 1969.

THE HISTIOCYTOMA, a benign tumor observed in the skin of young dogs, is a recognized pathologic entity, yet it remains an enigma in animal oncology because of its unique biologic and behavioral characteristics. Little more is known today about this canine dermal tumor than when Mulligan described the histopathology in 1949 and coined the name "histiocytoma" (*1*). The tumor is composed of round-to-oval cells, believed to be derived from connective tissue histiocytes. The cell population is usually monomorphic, unless necrosis and ulceration induce a secondary inflammatory response. Epidermal hyperplasia is often present. Some investigators have considered the cell population to be heterogeneous and have suggested that it is basically inflammatory rather than neoplastic (*2*).

Mulligan considered the histiocytoma and transmissible veneral tumor (TVT) to be the same tumor and used the name histiocytoma for both (*1*). Others have considered the histiocytoma to be an extragenital form of the TVT. Recent evidence supports a concept that the two tumors are separate entities despite some histologic resemblance. Cytogenetic studies have indicated that the karyotypes of cells of the histiocytoma

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] This study was supported by Public Health Service research grant CA 06897-03 from the National Cancer Institute and by funds provided by Bill 22-348 of the 1963 Connecticut Legislature for Research in Canine Diseases.

are those of the normal dog (*3*) while those of the TVT show a consistent abnormality (*3–5*). The two tumors also exhibit different biologic and epidemiologic characteristics (*6, 7*).

The canine histiocytoma appears to be unique in the dog; there are no known counterparts in other animals or in man, despite the frequency with which the name "histiocytoma" is applied to neoplastic and non-neoplastic diseases of other species. The so-called histiocytoma of primates, caused by the Yaba virus, is a benign cutaneous proliferative tumor composed of cells believed to be histiocytes; however, the cells of the lesion in primates are larger than those of the canine histiocytoma and the cytoplasm contains characteristic viral inclusions and lipid. The cells of the canine histiocytoma are smaller, and there are no lipid and no viral inclusions.

This report describes biologic, epizootiologic, and histopathologic characteristics of the canine histiocytoma based on studies performed in the Department of Animal Diseases, University of Connecticut.

MATERIALS AND METHODS

Canine skin tumors were submitted by veterinarians practicing in the State of Connecticut. Some were removed as incidental lesions during necropsies. Tumors were fixed in 10% formalin solution, embedded in paraffin, cut at 6 μ thickness, and stained with hematoxylin and eosin. Selected sections were stained with 0.1% aqueous toluidine blue, Verhoeff-van Gieson, Weigert's reticulum, Fontana silver nitrate, Unna-Pappenheim, and periodic acid-Schiff (PAS) to make differential diagnoses or to study cellular staining characteristics.

Tumors were classified according to the age, sex, and breed of the dog and size, duration, seasonal occurrence, and anatomic sites of occurrence.

RESULTS

During an 8-year period (1958–66), 376 canine histiocytomas were observed, of approximately 11,000 total accessions of histologic material from dogs.

No sex preference was noted. Fifty-three percent occurred in dogs younger than 2 years. The tumors were observed most frequently in late winter and early spring, with a peak occurrence in March. Approximately 80% measured less than 2.0 cm when removed. Clinical duration was short according to owners of dogs; most tumors had been present only 1–4 weeks at the time of excision. These observations are consistent with a relatively rapid rate of growth of these tumors.

As had been reported previously, boxer dogs appeared to be unusually susceptible to the histiocytoma (*8*). A previous survey of the same Connecticut dog population had indicated that boxer dogs comprised 3% of the dog population in the area (*9*). Twenty-one percent of 376 histiocytomas in this series occurred in boxers, a rate 7 times greater than expected. No other breed of dog or crossbreed had significantly increased rates of occurrence of histiocytomas.

Anatomic sites of involvement were of a characteristic distribution. Twenty-five percent occurred in the skin of the head, of which more than one-third involved ears or ear flaps. The next most frequent site was the skin of distal forelegs and forefeet, sites of 20% of tumors. The two locations accounted for 45% of all tumors, a small proportion of the total-body integument. Only 4% were observed in perigenital regions (prepuce, scrotum, perineum, and tail). None were in mucosa; all were in skin.

Histologically, tumor cells were usually located in the superficial dermis, adjacent to the basal layer of epithelium, and often appeared to be lined up in rows which "streamed off" the basal epithelium (figs. 1–3). The cell population was usually monomorphic; the cells were round-to-ovoid, with large, pale-staining nuclei and moderate amounts of pale blue-grey, poorly demarcated cytoplasm (fig. 4). From 3–8 mitotic cells were observed per high-power field. Hyperkeratinization of overlying epithelium and pseudoepitheliomatous hyperplasia were observed occasionally. This observation, plus varying degrees of mitotic activity of the basal epithelium (fig. 3), has been interpreted by some investigators to indicate that epithelial proliferation may be involved in the neoplastic process, or that the histiocytoma may in fact be an adnexal carcinoma. The opinion that epithelial cells are involved in the tumor, other than by occasionally demonstrating a reactive hyperplasia, has not been widely accepted.

The neoplastic cells were densely packed in the deeper layers of dermis, but were usually more loosely arranged nearer the epidermis. Necrosis and ulceration of the epithelium, which occurred in a high percentage of the lesions, often resulted in a gross appearance that suggested infectious granulomas. Clinically, damage to the overlying epithelium was often incident to self-trauma by licking or scratching.

Differential staining techniques were of little help in determining the origin and development of the tumor cells. Toluidine blue stains revealed an occasional mature tissue mast cell in the tumor, but the proliferating cells contained no metachromatic substances. Only occasional tumor cells, those with the most abundant cytoplasm, contained PAS-positive material. The Fontana stain was negative for melanin, and Weigert's stain indicated only a sparse scattering of reticulum fibers.

The histiocytoma was occasionally difficult to distinguish from poorly differentiated mastocytomas. The mastocytoma is also typically composed of proliferations of round or ovoid cells in the dermis, with invasion of the subcutis and occasionally of the underlying musculature. However, tumor mast cells are slightly larger than cells of the histiocytoma, and their cytoplasmic borders are distinct. Their cytoplasm often has a granular appearance with hematoxylin and eosin staining. Granules are obvious in cells stained with toluidine blue.

The rarely encountered cutaneous lymphomas may also cause difficulty in differential diagnosis. One boxer studied in our laboratory had multiple cutaneous lymphomas and apparently no other systemic involvement. The neoplastic lymphocytes and lymphoblasts appeared to arise in deeper layers of the dermis and were smaller than cells of histiocytomas. Cytoplasm was less abundant and nuclei were deeply stained in a manner characteristic of lymphocytes or lymphoblasts.

DISCUSSION

The cutaneous histiocytoma of the dog appears to be a distinct pathologic entity, with characteristic features that identify it and distinguish it from other "round cell" tumors of the skin of dogs. The monomorphic cell population, numerous mitotic figures, and pattern of growth suggest that the histiocytoma is a true neoplasm rather than an inflammatory reaction to an unknown agent. Metastases have not been reported. Multiple tumors occurred in less than 4% of dogs in our series.

The histiocytoma differs from most other similar canine neoplasms in that it affects primarily young dogs. Mature and older dogs are relatively resistant, which may indicate an acquired immunity. It appears that a dog recovering from a histiocytoma or from surgical excision will be resistant to the tumor. The TVT also induces immunity following recovery. Both tumors can undergo spontaneous regression, and both occur primarily in young dogs.

The epidemiologic features of the histiocytomas compared to the TVT are interesting. During the period of this study we observed no TVT's in the Department of Animal Diseases of the University of Connecticut. Veterinarians in areas in which TVT's occur frequently do not appear to be aware of cutaneous histiocytomas.

The etiology and pathogenesis of the cutaneous histiocytoma are not known. The behavioral and epizootiologic characteristics suggest an infectious agent. Successful transmission of the histiocytoma has not been reported. In contrast, the TVT can be readily transmitted experimentally, and of course by coital contacts.

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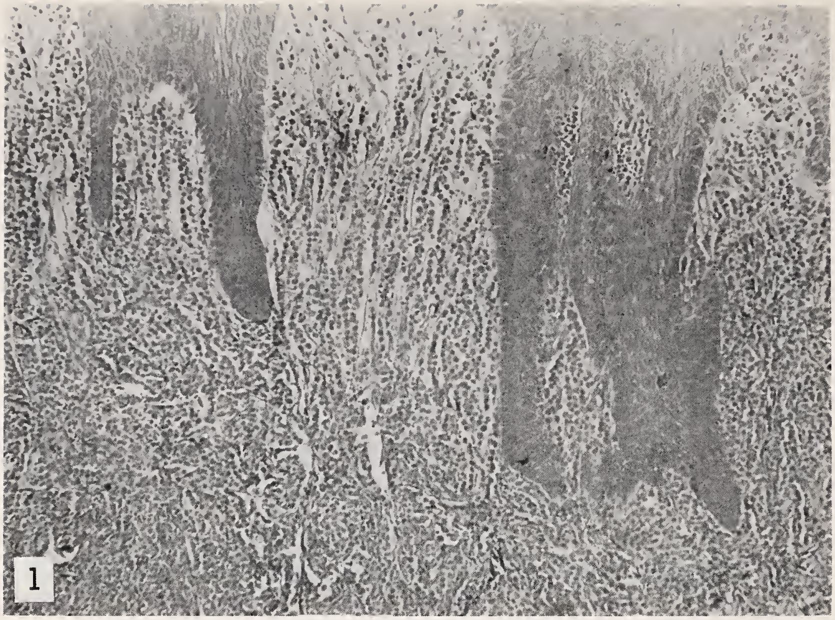
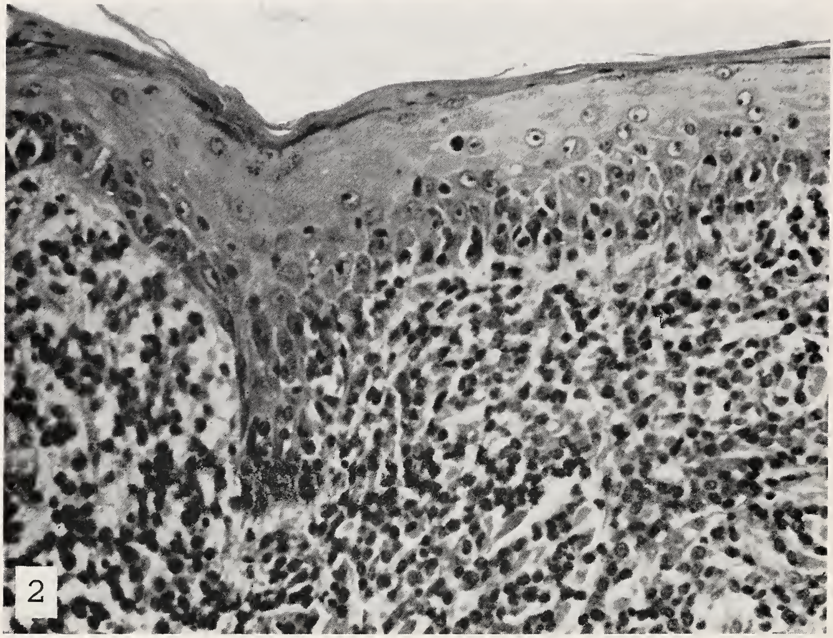
HEMATOPOIETIC NEOPLASMS

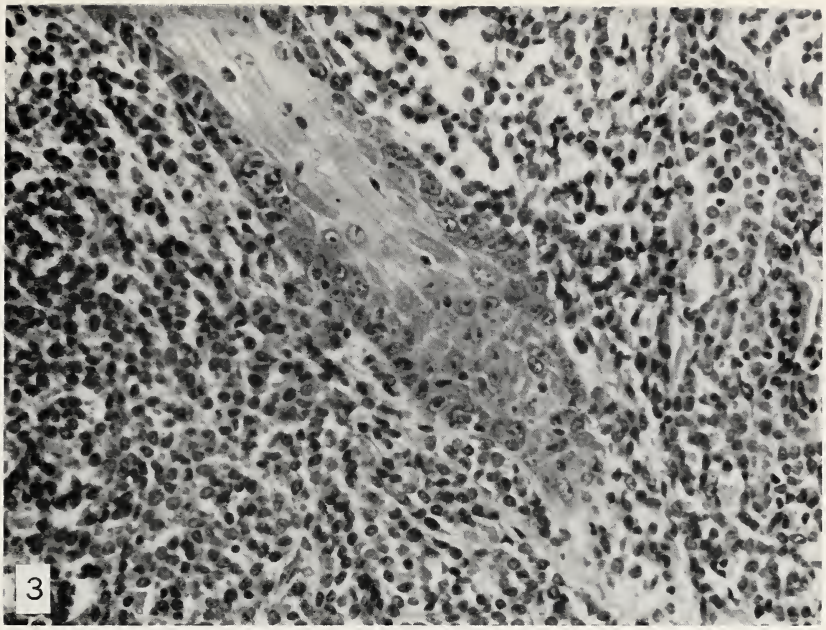




In figures 1–4, hematoxylin and eosin staining was used.

Figure 1.—Histiocytoma, skin, showing dense infiltrates of cells in the dermis. × 63

Figure 2.—Histiocytoma. Slightly higher magnification, illustrating relation of cells of the infiltrate to epidermal epithelium. × 160



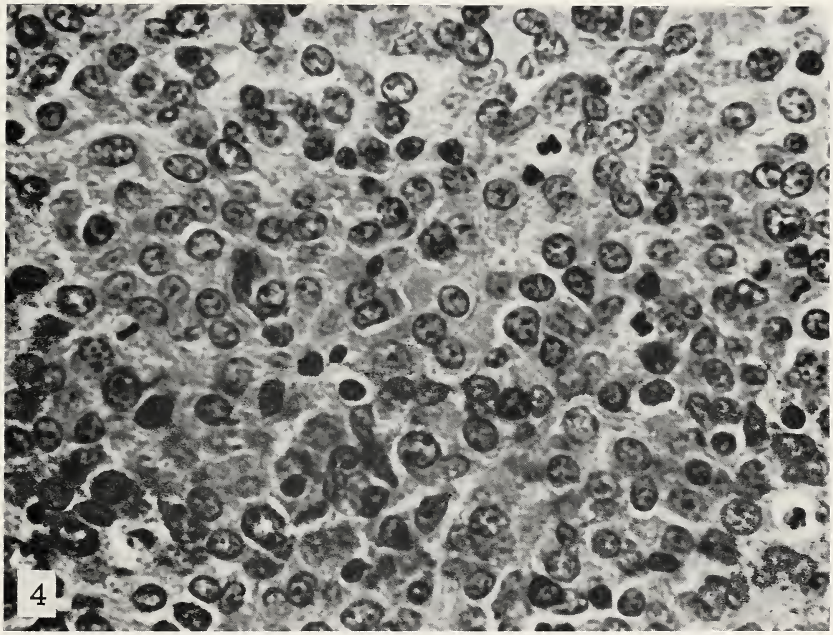


Figure 3.—Histiocytoma. Mitotic activity is apparent in the basal epithelium of a rete peg. × 160

Figure 4.—Histiocytoma, showing the characteristic monomorphic cell population. Nuclei are round-to-ovoid. Cytoplasm is abundant. Several mitotic cells are visible. × 400

Yaba Virus Tumors

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IN 1958, Bearcroft and Jamieson (*1*) first described an outbreak of subcutaneous tumors in rhesus (*Macaca mulata*) monkeys in Yaba, Nigeria. Subsequently, a viral etiology was established for the tumors and many reports have appeared in the literature describing the nature of the virus, host susceptibility, and pathogenesis of the lesion (*2–6*). The virus has received attention of comparative oncologists because it incites tumors. These tumors are described here because of their unique nature and the name which may cause confusion in comparative oncology because the term "histiocytoma" has also been applied to lesions of dogs and humans which are morphologically quite different. We emphasize that the term "tumor" is used in its broadest sense, and it is unclear whether the reactive lesion which characterizes infection with the Yaba virus should be considered a true neoplasm.

The salient features of the Yaba tumor are that a DNA virus, probably a member of the pox group, produces characteristic subcutaneous growths on the face and distal portions of the limbs in some, but not all, species of nonhuman primates and in man. Tumors are morphologically similar in all susceptible species. They regress spontaneously as host immunity develops, and reinoculation of previously infected animals fails to elicit tumorigenesis.

Gross examination reveals variable-sized lesions (up to 5–8 cm in diameter), which have usually risen above the skin surface and are often ulcerated. In spontaneous cases, satellite tumors may radiate from the original mass, but no distant metastases have been observed. Hemorrhage may be a prominent feature, since lesions are easily traumatized.

Histologic examination of hematoxylin-and-eosin-stained sections of a mature tumor reveals a uniform pattern which consists of large pleomorphic "histiocytic" cells loosely arranged in a generous vascular network containing occasional eosinophils and plasma cells (figs. 1 and 2).

The growth is not encapsulated, and the proliferating histiocytes infiltrate the surrounding tissue, apparently following paths of least resistance,

337-730—69——40

which in the subcutis are the pre-existing vascular-connective tissue planes (figs. 3 and 4). Occasional remnants of mature, probably pre-existing collagen fibers are deep in the tumor mass. The histiocytes contain large, round-to-oval vesicular nuclei, characteristically, in which there are one or more large, prominent, basophilic nucleoli. There is abundant eosinophilic cytoplasm, with a fibrillar or granular appearance associated with the presence of lipids. The cytoplasm is often shrunken or pulled away from the cell wall, giving a loculated appearance (fig. 2). The diagnostic features of the cell (and this tumor) are the presence of large (2–5 μ diameter), single or multiple, well-defined, dark eosinophilic, intracytoplasmic inclusions (figs. 5 and 6). Mitoses are rare. The only variations from this description are in peripheral areas or near the eroded surfaces in which granulocytes may be more prominent.

As the lesion enlarges, well-defined degenerative changes begin which eventually overshadow the proliferative phase and finally result in regression of the tumor. The regressive phase is characterized by degenerative changes in individual cells as noted by Sproul *et al.* (*3*), and definite zones of necrosis are not usually observed except near ulcerated surfaces, although Niven *et al.* (*6*) described a definite zone of necrosis in the center of a tumor of an experimentally inoculated monkey. The regressive phase includes an infiltration of plasma cells and lymphocytes which are first observed as perivascular collars and later may be diffusely scattered throughout the lesion, usually becoming quite prominent. Polymorphonuclear leukocytes digest degenerating histiocytes (fig. 7).

An interesting feature of the regressive phase is the appearance of variable numbers of large multinucleated cells which are believed to be end stages of incomplete tumor cell division (figs. 7 and 8) (*3*). Consistent with this origin is the fact that the giant cells contain characteristic nuclei and, of more importance, the intracytoplasmic inclusions (fig. 8).

As summarized by Sproul *et al.* (*3*), regression appears to be a process of tumor cell disintegration and there is little residual scarring of the skin.

In the past, there was some question as to whether the essential cell was fibroblastic (*6*) or histiocytic in origin. Studies of lesions from virus-inoculated susceptible hosts which were serially sacrificed have indicated that the origin of the large pleomorphic cell appeared to be the tissue histiocyte and therefore this lesion has been referred to as a histiocytoma (*3*). Despite use of this name, there is little histologic or morphologic resemblance to the cutaneous histiocytoma of dogs.

In my experience, pathologists who are not familiar with the entity make variable diagnoses including: 1) granular cell tumor (granular cell myoblastoma); 2) fibrous xanthoma; and 3) alveolar soft part sarcoma. It should be pointed out that these diagnoses are ordinarily made with reservation because the intracytoplasmic inclusions are usually prominent. Once a pathologist has seen a Yaba virus-induced tumor he can recognize them easily.

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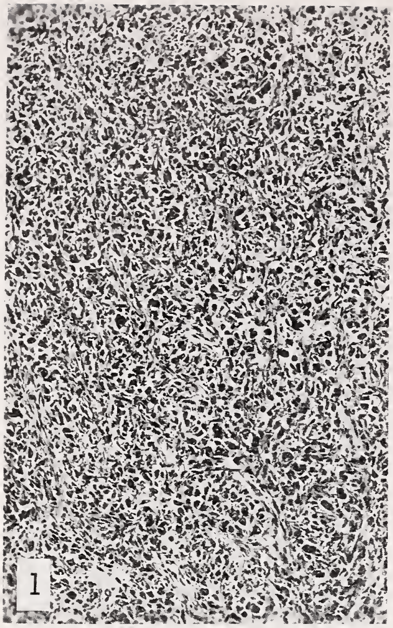
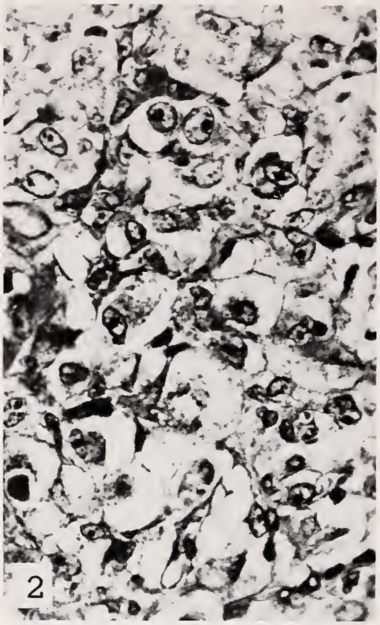
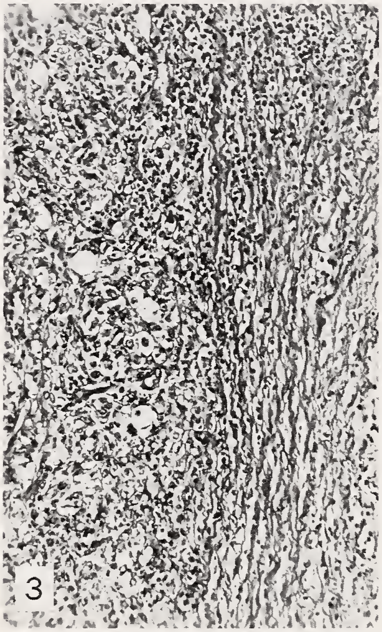






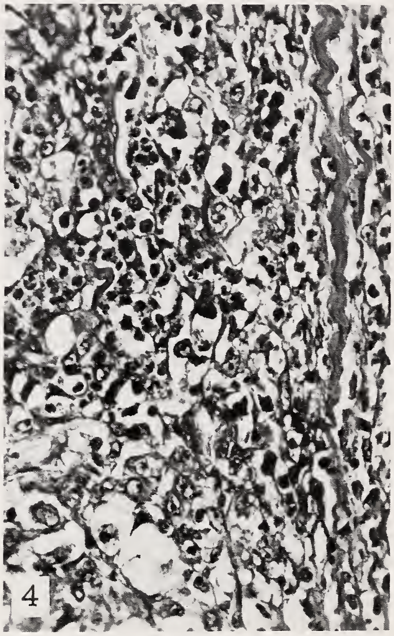
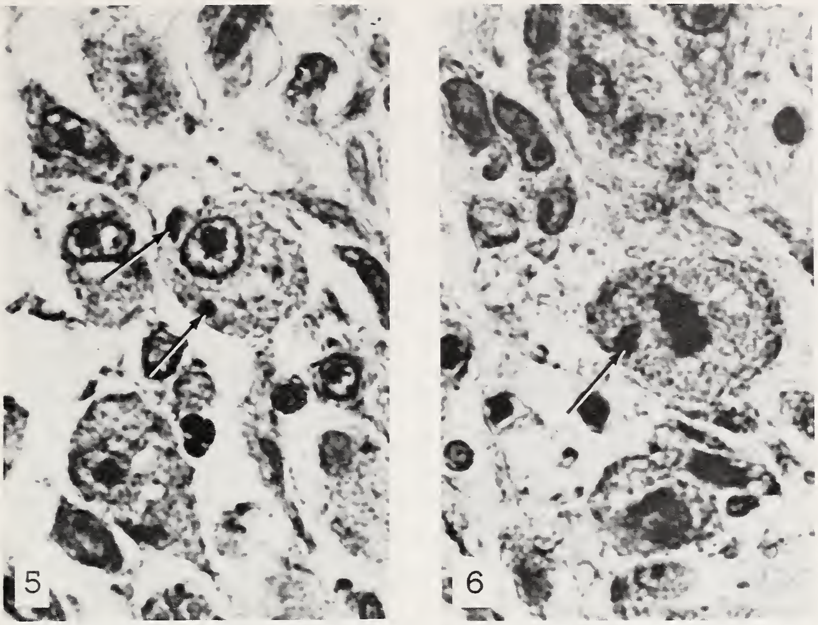


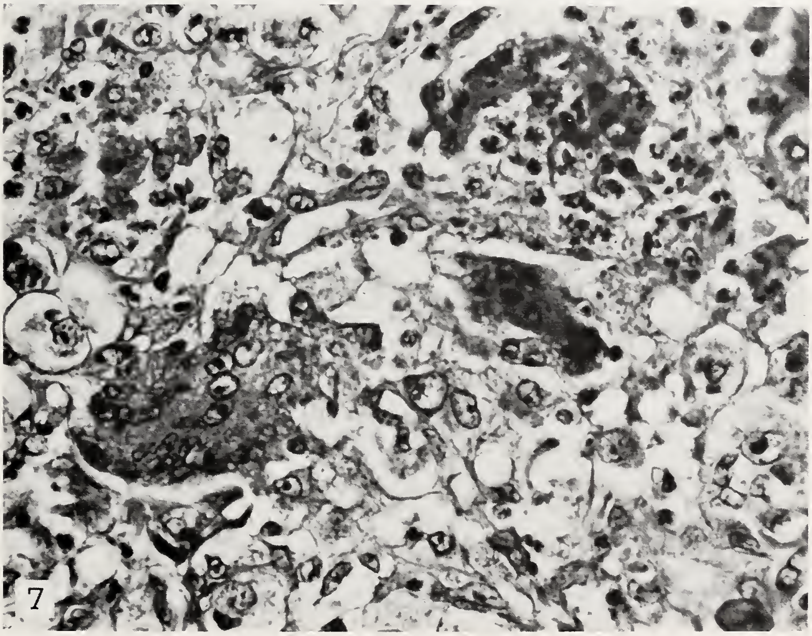
FIGURE 1.—Representative section of Yaba monkey virus tumor during proliferative stage. AFIP negative 68-8115-4. × 60

FIGURE 2.—Higher magnification of figure 1. *Note* histiocytic appearance with typical locular formation of the cytoplasm. AFIP negative 68-8115-4. × 350

FIGURE 3.—Edge of lesion with tumor cells freely infiltrating the pre-existing connective tissue. *Note* zone of leukocytes between tumor and connective tissue. AFIP negative 68-8115-1. × 100

FIGURE 4.—Higher magnification of figure 3. AFIP negative 68-8115-1. × 260





Figures 5 and 6.—Examples of tumor cells containing prominent intracytoplasmic inclusions (*arrows*). AFIP negative 68-8115-3. × 1000

Figure 7.—Necrosis of giant cell with phagocytosis by numerous polymorphonuclear leukocytes (*top*). *Note* presence of viable multinucleated giant cell (*left*). AFIP negative 68-7672. × 450

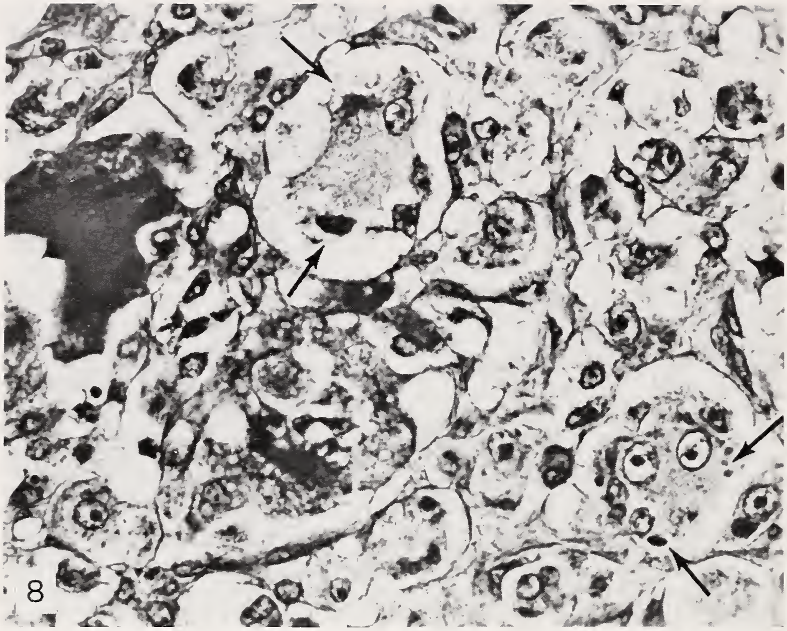


Figure 8.—Multinucleated giant cells containing prominent intracytoplasmic inclusions (*arrows*). AFIP negative 68-8115-2. × 450.

Hamster Lymphoma, TM [1]

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A TUMOR measuring 18 × 18 × 5 mm was taken from the upper lip of a 2-year-old male Syrian hamster on January 9, 1959. Histologically, we called it a reticulum cell sarcoma and labeled it TM. It was carried through 164 generations by subcutaneous trocar transplantation. This tumor has been of exceptional interest, since a virus has not been found to be associated with it and it is contagious in that nontumorous hamsters placed with tumorous hamsters will develop the tumor (*1*). The transfer is through cannibalism.

In most instances the eating of the tumor results in its development first in the larynx. It never involves the gastrointestinal tract directly. Its predilection for the larynx and how it gets there have not been explained. The experimental transfer of tumor by feeding has been previously reported (*2*, *3*).

TM has a characteristic male karyotype which is retained regardless of method of transfer, by feeding or subcutaneous transplant, and regardless of the sex of the recipient. The transfer of tumor is by the transfer of cells (*4*).

TM can also be transferred by the mosquito *Aëdes aegypti* (*5*, *6*). Tumor cells are present in the bloodstream of a hamster within 5 days of subcutaneous transplant. Terminally, there is a marked leukemia with a white blood cell count often of more than 100,000. Two percent of the mosquitos which feed on such a leukemic animal will transmit the tumor if the feeding is interrupted and then completed on a nontumorous animal. The unique male karyotype of the tumor is maintained from host to recipient regardless of sex. This indicates that transmission is by means of transfer of tumor cells and not of some other oncogenic agent. To our knowledge, this is the first time that such a mechanism, *i.e.*, transfer of mammalian tumor cells through an insect vector, has been demonstrated.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

It is important to find the extent and consequences of such transfer of cells in nature.

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The Chediak-Higashi Anomaly—an Evolutionary Concept of Disease[1]

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> Take twenty or more eggs and let them be incubated by two or more hens
>
> Then each day from the second to that of hatching, remove an egg, break it and examine it
>
> You will find exactly as I say, for the nature of the hen can be likened to that of man.
>
> Anonymous Greek, 350 B.C. (*1*)

THIS bit of philosophy is some 2,300 years old. Although the ideas expressed are recognized cornerstones of sciences of embryology, anatomy, physiology, biochemistry, and genetics, the fullest potential of the phylogenetic approach has not been recognized by students of disease. The Chediak-Higashi anomaly is an instructive disease model for the comparative oncologist who is concerned with basic biologic processes, particularly those which suggest associations of infectious and neoplastic diseases.

The Chediak-Higashi anomaly was first described in human patients by Beguez-Cesar in 1943 (*2*), although discovery has been credited to Chediak (*3*) and Higashi (*4*). Hematologists first noted the enlarged cytoplasmic granules of polymorphonuclear leukocytes, eosinophils, and lymphocytes in blood of affected individuals. The enlarged granules cannot be distinguished by tinctorial and histochemical methods from cytoplasmic granules of leukocytes of normal individuals. The defect also occurs in cells other than those of the blood and apparently results in decreased pigmentation of hair, skin, and iris. In humans, the defect is inherited as an autosomal recessive trait.

The structural abnormalities of granulocytes are associated with increased susceptibility to infections, particularly those caused by bacteria (*5*). Children with the Chediak-Higashi anomaly usually die of overwhelming bacterial infections by the age of 7 or 8 years, although occasionally individuals have survived into the teens. Proliferative lesions of the hematopoietic system, believed by some investigators to be lymphomas or leukemia, have been described in a few individuals with the defect.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

With fewer than 50 human cases reported, the Chediak-Higashi anomaly remained little more than a medical curiosity until 1963 when Padgett *et al.* (*6*) and Leader *et al.* (*7*) reported observations of a similar syndrome in mink. A definite genetic basis for the defect of mink has been established. Mink carrying the anomaly are more susceptible to Aleutian disease—an infectious disease presumably caused by a virus—than those without the defect. The hematologic anomaly provides a convenient method for identifying susceptible mink. Padgett has reviewed details of this syndrome (*8*). Although an immunoproliferative process occurs in mink with Aleutian disease, lymphomas or other neoplasms have not been observed.

The discovery of an animal model allowed investigations into the fundamental nature of the Chediak-Higashi anomaly. By electron microscopic studies, Lutzner confirmed the abnormal formation of lysosomes not only in leukocytes but in cells of other types (*9*). There is evidence that the abnormal lysosomal activity is related to defective capacity to deal with infectious agents and to the process of oncogenesis (*10*).

The Chediak-Higashi syndrome has also been observed in cattle (*5*) and in mice (*11*). Cattle with the syndrome are also abnormally susceptible to bacterial infections.

From the point of view of evolution, the lysosomal defect of the Chediak-Higashi syndrome is intriguing because it occurs in species widely separated on the evolutionary scale. Text-figure 1 shows that at least 60 million years have passed since ancestors existed that were common to mustelids, ungulates, and primates. It is not known whether the Chediak-Higashi defect has been carried as a genetic load throughout the phylogenetic history of the animals involved and is only occasionally expressed in the homozygous state, or whether it represents a labile genetic locus which has periodically undergone mutation possibly because of some environmental influence. The latter explanation is more probable. The genetic defect appears to involve one gene locus which is multisystemic in its expression. The abnormaltiy is a biochemical lesion of a fundamental nature that affects processes shared by cells of diverse specializations.

Comparative pathologists must be alert to opportunities to identify additional diseases in animals which may provide insights into fundamental biologic and pathologic processes.

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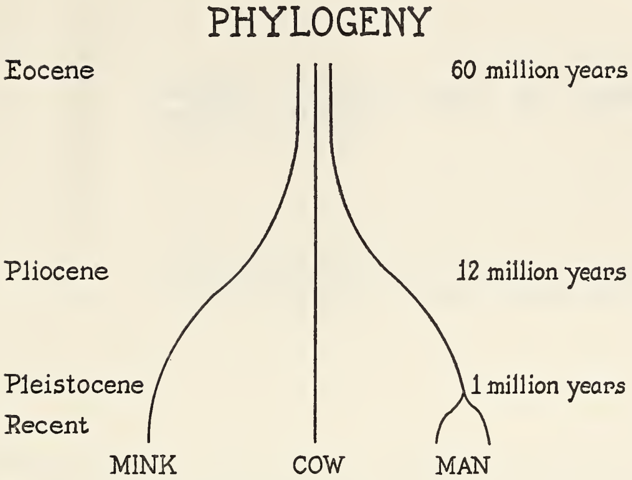

Text-figure 1.—Diagram illustrating theoretical evolutionary period since separation of man, mink, and cow.

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Biologic Behavior—Bases for Comparative Studies of Disease[1]

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THIS SYMPOSIUM was organized as a means of bringing people together to discuss hematopoietic neoplasms on a comparative basis. There is a difference of opinion as to what constitutes a proper comparative study. Some investigators believe that true comparisons can be made between diseases of man that also affect lower animals. Others accept comparisons between individuals of two or more genera suffering the same disease, whether or not one of them is *Homo*. It was gratifying to discover the definition of comparative pathology formulated at the Workshop Conference sponsored by the National Institute of General Medical Sciences: "a study of phenomena basic to the diseases of all species (*1*)." Using this definition, we can discuss the comparative morphology of neoplasms that occur in lower animals but not in man, even though we hope that mankind may eventually benefit from knowledge developed through such comparisons. Indeed, as Harold Stewart pointed out earlier in this Conference, we have as yet to elucidate a single human neoplasm from comparative observations of animal tumors.

The canine transmissible venereal tumor and cutaneous histiocytoma of dogs illustrate the problems of identifying tumors by use of cell morphology as the sole criteria. If the type of cell composing a tumor is disputed, we must seek other means for differentiation, such as age, sex, genetic endowment of the host, anatomic sites of predilection, transmissibility, immune response, spontaneous regression, chromosome karyotype, and other factors. When morphologic distinctions are subtle and tumors of similar appearance share some generic features, they must be separated by any possible means, if such separation can be justified.

Of interest to oncologists is the unique situation of the canine venereal tumor; the neoplastic cells are transplanted mechanically on the mucous membranes of the host, yet they are sufficiently unlike the host's tissue to evoke an antibody response. The transmissible reticulum cell sarcoma of the hamster apparently is not associated with an exciting virus or other known causative agent, yet the tumor cells readily implant in a new host following cannibalism, mosquito bite, or injection of cells. The tumor maintains a male karyotype regardless of the sex of the recipient hamster and a chromosome number unlike that of the hamster. That a canine lymphoma can be transplanted by inoculation of whole cells into pups *in utero* is of special interest to investigators of human hematopoietic neoplasms, because the canine and human lymphosarcomas are similar morphologically and biologically.

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Studies of both neoplastic and non-neoplastic diseases of animals illustrate important biologic principles that are common to many species and that must be kept in mind when one searches for causes of both hematopoietic and nonhematopoietic tumors. The morphologist must continue to be aware of the roles of virologists, immunologists, geneticists, and other basic scientists in elucidating mechanisms of disease processes that are basic to all species.

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A Classification of Lymphoid Neoplasms of Domestic Mammals [1]

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SUMMARY

From our study of malignant neoplasms in animals, it would appear that the pathogenesis of the major disease, which we term "lymphosarcoma," is essentially similar in the five species described. The condition is primarily a neoplasm involving lymphoid organs in multicentric distribution with variable infiltration of other organs. Leukemia is present in approximately 10% of cases. Thus hematological examination is of less value in the original diagnosis and assessment of the stage and prognosis of the disease in animals than it is in man. Since we have found no correlation between the duration of the disease and the type of cell and mitotic rate predominating in the tumors, cytological criteria would also seem to be of limited value. Therefore, we propose that a system of classification for use in experimental and epidemiological studies should include categories dealing with the anatomical distribution of the major lesions. This system would allow a clear-cut grouping of the great majority of cases into two or three main forms in each species. The disease in its various anatomical forms is possibly a single biological entity, however, and in cats we have produced experimentally the alimentary, multicentric, and thymic forms by transmission of material from a single cat with thymic lymphosarcoma. Whether the apparent difference in epidemiological features and the age incidence in bovine lymphosarcoma in different countries are indicative of a different disease is not yet known. We have not seen spontaneous cases of acute lymphoblastic leukemia, though we have produced this form of the disease experimentally in cats and it has been reported in the field in this species. Hodgkin's disease has not been found in our hospital series, and the relatively rare hematopoietic neoplasms examined include disseminated myelomatosis, myeloproliferative disorders, granulocytic leukemia, and mast cell reticulosis.—Nat Cancer Inst Monogr 32: 343–353, 1969.

AS A preliminary exercise in producing a classification of lymphoid neoplasms in domestic mammals for the World Health Organization, we have reviewed a series of 483 cases collected in our laboratory from Great

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[1] Read at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

Britain. References (*1–11*) refer to publications in which members of this series have been reported in more detail; no general literature review is included in the present paper. We hope to amend or extend the suggested classification after consultation with other workers in this field in different parts of the world. The classification proposed is therefore tentative and is intended to illuminate some difficulties arising if one tries to fit animal lymphoid neoplasms into the framework evolved for human leukemias. The latter has great strength diagnostically and prognostically, although perhaps less biologically. The purpose of this work in animals is to produce a system that will be meaningful in epidemiological and experimental work.

The major criteria we have selected and reviewed for the proposed classification are the cytology, mitotic rate, age incidence, anatomical distribution of the major lesions, and the presence or absence of leukemia.

The series consists of 152 dogs, 38 cats, 167 cattle, 34 sheep, and 92 pigs. The dogs, cats, and 60 of the cattle were hospital cases and hence had been examined clinically, hematologically, and pathologically; most of the others were in an abattoir survey. Thus, in the dog and cat, we have more information on such aspects as hematological features and presenting signs, while in cattle, sheep, and pigs we have more accurate relative and absolute incidence figures, since the denominators of the total numbers of live and slaughtered animals in Britain are known. The major lymphoid neoplasm in these species is characterized by the infiltration of lymph nodes and other organs by malignant cells of the lymphoid series; usually the disease is confined to the solid tissues, and the high level of circulating lymphocytes is relatively uncommon. For these reasons, we consider the term "lymphosarcoma" to most accurately describe the disease as it occurs in domestic mammals. However, the terms "lymphatic leukosis" have been used for many years, especially on the Continent of Europe. In general biological nomenclature, "leukemia" is currently used to describe the disease complex rather than a strict hematological situation; "murine leukemia" is the name generally used for the complex as seen in mice, which is very similar to that in domestic animals.

Cytologically, most animal tumors are lymphosarcomas, with lymphoblasts and large lymphocytes predominating. A smaller proportion has more mature cells of the lymphoid series consisting mainly of medium-sized lymphocytes. In contrast to the situation in man, no consistent relation has been demonstrated between the type of cell predominating in a lymphosarcoma and the duration of the overt disease. Further, the maturity of cell type may vary in different lymph nodes in the same affected animal and there is no consistent tendency for the cells to become progressively more immature as the disease enters its terminal phase. Acute and chronic leukemias, as seen in man, occur only rarely in animals. Stem cell and acute lymphoblastic leukemias have been produced experimentally in cats and have been seen occasionally in the field.

In all domestic mammals studied, lymphoblastic or immature lymphocytic lymphosarcoma is the major lymphoid neoplasm, regardless of the age of the affected animal or the anatomical distribution of the lesions. Leukemia is relatively uncommon in the disease and is found in approximately 10% of animals with lymphosarcoma, though this varies between species and within types. There is no evidence of regular progressive development of leukemia within the course of the disease.

In the proposed system, we have classified lymphosarcoma on a morbid anatomical basis. It is not implied, however, that morphological variants are separate biological entities, and lymphosarcoma may be essentially one disease presenting in certain different anatomical distributions. For instance, in cats, the alimentary, multicentric, and thymic forms have been produced experimentally by transmission from a cat with thymic lymphosarcoma.

The factors favoring a morbid anatomical classification are:

1) Cytological features have not been proved related to the duration of the overt disease, and most animal lymphosarcomas are of lymphoblastic or immature lymphocytic type.
2) The anatomical variants, at least in cattle, have epidemiological significance. In enzootic bovine lymphosarcoma, the disease is almost always multicentric in distribution, whereas in sporadic lymphosarcoma, a high incidence of the thymic form is seen (49% in our series). Further, there are distinct age differences in the incidence of the enzootic and sporadic conditions which are related to the anatomical distribution: Enzootic lymphosarcoma affects adults almost exclusively, whereas sporadic lymphosarcoma has a high incidence in young animals that also show a predominance of thymic lymphosarcoma. In our series, 44% occurred in cattle less than 2 years old and more than half of the juvenile cases were thymic.
3) Lymphosarcoma occurs in certain distinctive distributions, giving rise to recognized clinicopathological syndromes. The morbid anatomical classification, therefore, has a useful descriptive function.

Following are the definitions of terms used in the proposed classification:

Multicentric lymphosarcoma: disseminated lymphosarcoma in which bilateral enlargement of superficial lymph nodes and variable infiltration of other organs are usual. The organs most commonly involved are the liver, spleen, kidneys, lungs, myocardium, gastrointestinal tract, and bone marrow.

Thymic lymphosarcoma: lymphosarcoma which appears to start or grow most rapidly in the thymus. The thymus may be the only obviously affected site, or the condition may be disseminated with lymph node involvement and infiltration of other organs. The major lesion is always in the thymus, which becomes totally replaced by the neoplasm.

Alimentary lymphosarcoma: lymphosarcoma in which the major lesions occur in the gastrointestinal tract and mesenteric lymph nodes. One of these sites may be affected, though usually both are involved. The superficial lymph nodes are not affected, and splenomegaly is unusual. The liver is infiltrated in 50% of cases, but other organs are only rarely involved.

The skin form: disseminated lymphosarcoma in which infiltration of the skin and subcutaneous tissue produces nodular thickening and loss of hair. There is usually an associated multicentric complex.

Leukemia: absolute lymphocyte count greater than 30,000/mm^3 with or without circulating lymphoblasts, or a count of less than 30,000/mm^3 with an abnormally high proportion of one cell type.

LYMPHOSARCOMA IN THE DOG

Most lymphosarcomas in the dog occurred in one of two main morbid anatomical forms.

1) *Multicentric form.*—There was bilateral symmetrical enlargement of most or all of the superficial lymph nodes, together with involvement of the liver, the spleen, and most of the deeper lymph nodes. Other organs commonly infiltrated included the gastrointestinal tract, kidneys, lung, heart, tonsils, pancreas, and bone marrow. Occasionally the skin, eyes, and skeletal muscle were affected, and in one instance, the spinal cord was invaded.

2) *Alimentary form.*—The superficial lymph nodes were not enlarged and splenomegaly was seldom found. The major lesions were in the gastrointestinal tract and mesenteric lymph node group; one of these sites was sometimes involved alone, though usually both were affected. Occasionally, infiltration of other organs occurred in the alimentary form of the disease. The multicentric and alimentary forms each had an incidence of 45% of all dog lymphosarcomas. In the remaining 10%, the lesion distribution did not conform to either group. The distribution of the main lesions in multicentric and alimentary lymphosarcoma is shown in table 1.

The incidence of canine lymphosarcoma was greatest between 5 and 11 years, with 80% of all cases occurring in this age group. However, the disease was found less commonly in dogs of any age. In our series, there was no significant breed or sex incidence.

Leukemia was found in only 13 dogs in the series. Leukemia in the few animals which could be studied over a period was found to be inconstant and there was no evidence of any progressive development of leukemia within the course of the disease.

A study of the cells in normal dog lymph nodes and in lymphosarcomatous lymph nodes revealed that the types of cells found in the disease were essentially the same as those in normal nodes, differing only quantitatively in arrangement and in mitotic activity. The cells of the normal dog lymph node were similar to those of the normal human lymph node. The cells identified were the lymphoblast, large lymphocyte, medium lymphocyte, small lymphocyte, the plasma cell series, primitive reticular cells, and reticuloendothelial cells. The neoplastic lymph nodes were examined to identify the predominant cell type in the tumor and to estimate the mitotic rate. In 60 of 109 cases, the dominant cell was the large lymphocyte or one of the more immature cells of the lymphoid series; this was designated the "immature cell group." In 42 cases, the

Table 1.—Distribution of the main lesions in 122 cases of lymphosarcoma in the dog

Site	Multicentric	Alimentary
Lymph nodes		
Superficial	61	0
Thoracic	42	6
Anterior sternal	—	14
Mesenteric	49	50
Other abdominal	56	18
Liver	50	38
Spleen	52	5
Alimentary tract	19	47
Kidney	26	12
Lung	14	6
Heart	11	1
Tonsil	25	1
Marrow	14	1
No. of dogs examined	61	65

tumor was composed of more mature cells, with medium and large lymphocytes predominating; this was designated the "mixed or mature cell group." There was no difference in the cell types in multicentric and alimentary forms of lymphosarcoma. Mitotic activity was greater in those tumors composed of more immature cells. In 48 of 60 immature cell tumors, there was a high or very high mitotic rate, while in 29 of 38 mixed or mature cell tumors, the mitotic rate was moderate or low; there was a highly significant difference between the mitotic activity in these two groups. There was no relation between the age of the dog and the type of cell in the tumor. Evidence of a relationship between the cell type and the duration of the overt disease was sought; no such correlation was found.

We conclude that lymphosarcoma in the dog generally occurs in a multicentric or alimentary distribution and affects dogs of middle age, usually without evidence of leukemia. The cells of the tumor may be lymphoblasts, large lymphocytes with a high mitotic rate, or more mature lymphoid cells with many medium and large lymphocytes having a moderate or low mitotic rate.

LYMPHOSARCOMA IN THE CAT

In the cat, lymphosarcoma presented in three main morbid anatomical forms.

1) *Multicentric form.*—This form was similar to that occurring in the dog. 2) *Alimentary form.*—This form also corresponded to that of the dog. 3) *Thymic form.*—The major lesion was a large mass replacing the thoracic

thymus. In some cases, the thoracic thymus was the only site affected, but in others several lymph nodes and organs were involved to a lesser degree as well as the thymus. A small proportion did not conform to this grouping and the sites affected were variable. The distribution of the major lesions in 48 feline lymphosarcomas is shown in table 2. The alimentary form was the most common, accounting for 50% of all cases. In this condition, one or more tumors were found in the wall of the intestine, at any level, associated with gross enlargement of the main or posterior mesenteric lymph nodes; the heart and kidneys were also fairly commonly infiltrated.

TABLE 2.—Distribution of main lesions in 48 cases of lymphosarcoma in the cat

	Multicentric	Thymic	Alimentary	Others	Total
Superficial lymph nodes	14	—	—	1	15
Thoracic lymph nodes	9	—	—	—	9
Anterior sternal lymph nodes	1	—	5	1	7
Mesenteric lymph nodes	13	—	19	—	32
Other abdominal lymph nodes	12	—	5	1	18
Thymic mass	5	5	—	—	10
Liver	7	2	15	4	28
Spleen	8	1	5	—	14
Intestine	2	1	18	—	21
Kidneys	5	2	9	1	17
Lungs	3	1	2	2	8
Heart	2	1	—	—	3
Tonsils	3	—	—	—	3
Marrow	—	—	1	1	2
Pancreas	—	—	2	—	2
No. of cats examined	14	5	24	5	48

We have found no evidence of any consistent age incidence, and the disease was seen in cats ranging from 1–16 years old. There was no breed or sex predisposition.

As in the dog, leukemia was not common. In 30 cats examined hematologically, leukemia was present in only 2, whereas an absolute lymphopenia was found in 5. In the 2 leukemic cats, circulating lymphoblasts were present in peripheral blood. Normocytic normochromic anemia was often found in lymphosarcoma, and half the animals examined had a packed cell volume below 30%, with parallel reductions in hemoglobin concentration and total erythrocyte counts. The erythrocyte sedimentation rate was usually high and negatively correlated with the total erythrocyte count. Differential white cell counts in 30 cats are shown in table 3.

The cells composing any given tumor were fairly uniform, and the range observed in lymphosarcoma varied from stem cells to mature lymphocytes. There was no difference in the tumor cells found in the various anatomical types of lymphosarcoma and no correlation between the predominant cell and the duration of the overt disease. As in the dog, it was not demonstrated that cytological features had any prognostic value. We have not seen any field cases of acute lymphoblastic leukemia, as described by Holzworth (*6*). However, we have produced this form of the disease experimentally in cats infected as newborns with cell-free extracts of lymphosarcoma tissue. It is possible that acute spontaneous cases would not be identified and submitted to our hospital for examination.

Our results indicate that lymphosarcoma in the cat occurs at any age and usually presents in either the alimentary, multicentric, or thymic form. Leukemia is uncommon and the predominant tumor cell may vary from stem cell to mature lymphocyte.

TABLE 3.—Circulating leukocytes in 30 cats with lymphosarcoma

White blood cells ($10^3/mm^3$)	Number of samples	Neutrophils ($10^3/mm^3$)	Number of samples	Lymphocytes ($10^3/mm^3$)	Number of samples
<5	5				
5–	26	<5	7	<2. 5	16
25–	6	5–	23	2. 5–	17
50–	4	15–	9	7. 5–	3
75–	2	25–		15. 0–	2
100+		35–		30. 0–	1
		45–	2	60. 0	3
		55+	1	90. 0+	
Total	43		42		42

LYMPHOSARCOMA IN CATTLE

Lymphosarcoma in cattle was in three main anatomical forms.

1) *Multicentric form.*—This form was the same as that described in small animals. 2) *Thymic form.*—This form also was the same as that described in small animals. 3) *Skin form.*—This was a disseminated lymphosarcoma with infiltration of the skin and subcutaneous tissues producing a nodular thickening and loss of hair. In a series of 167 cases, 49% were thymic, 48% multicentric, and 3% were of the skin type.

The age incidence found in bovine lymphosarcoma in Britain was of particular interest because of certain differences when compared with the disease in enzootic areas. In our series, 74 of 167 bovine cases (44%) occurred in animals less than 2 years old and 114 (68%) occurred in animals less than 4 years old. In our experience, lymphosarcoma is fairly frequent in neonatal calves and the present group included 5 calves less than 2 months old. Also, in the British series, the thymic form of lymphosarcoma predominated in animals 2–3 years old. The age, sex, and anatomical distribution in the 167 cattle are shown in table 4. There was no evidence of any sex predisposition to lymphosarcoma. Approximately equal numbers of male and female animals were affected under 2 years

TABLE 4.—Age and type distribution in 167 cases of bovine lymphosarcoma in Britain

Age (yr)	Male	Female	Male neuter	Total	Type		
					Multicentric	Thymic	Skin
<1	13	16	0	29	16	13	0
2	1	26	18	45	18	26	1
3	1	21	14	36	13	21	2
4	1	2	1	4	2	2	0
>4	1	51	1	53	32	20	1
Total	17	116	34	167	81	82	4

of age, and the higher incidence in female adults reflects the fact that most adult cattle are cows. There was no particular breed incidence and the disease occurred in all the common British breeds.

In multicentric lymphosarcoma, the most frequently infiltrated internal organs were the spleen, liver, kidneys, and lungs. In calves, the multicentric form had an additional characteristic: extensive replacement of the bone marrow. Thymic lymphosarcoma involved only the thymus in some animals, but when the disease was disseminated, the same organs tended to be affected as in the multicentric form.

To determine whether the enzootic form of bovine lymphosarcoma is present in Britain, follow-up investigations were carried out in every case by veterinary officers of the Ministry of Agriculture. The farm of origin was visited and live animals were inspected for any evidence of disease. The farmer and his local veterinary surgeon were interviewed to find whether the disease had ever previously occurred on the farm. Never did these investigations reveal that lymphosarcoma was present in other animals or that it had occurred before on the farm. Blood samples were examined from cattle in 31 herds from which adult lymphosarcoma cases originated, and differential cell counts were carried out. These examinations showed no evidence of lymphocytosis in the apparently normal adults in any herd.

Generally, the cells within individual tumors appeared fairly uniform, and the range observed varied from lymphoblasts to mature lymphocytes. Clinical examination of affected cattle at our hospital has shown that leukemia is not often present during the disease, being found in less than 10% of cases.

To summarize, bovine lymphosarcoma as found in Britain appears to be an entirely sporadic disease affecting animals of any age but occurring most commonly in cattle less than 4 years old. The condition is usually seen in either the thymic or multicentric form and occasionally in the skin form. It is most frequent in the thymic form and has its highest incidence in adolescent animals. The cell type tends to be fairly uniform in individual tumors, varying from lymphoblast to mature lymphocyte. Leukemia is seldom a feature and lymphocytosis is not present in healthy animals in the herd of origin.

LYMPHOSARCOMA IN SHEEP

Lymphosarcoma in sheep was found in the *multicentric*, *thymic*, and *skin* forms, corresponding to the distribution in bovine lymphosarcoma. In sheep, the multicentric form predominated, accounting for 75% of all cases. The remainder in our series were equally divided between thymic and skin types. In disseminated cases, the organs usually infiltrated were liver, spleen, kidneys, and lungs; skeletal muscle, myocardium, and peritoneum were affected less regularly.

Our 44 cases were found during an abattoir survey of neoplasms and most were mature or old ewes; the youngest affected sheep was age 8 months. Due to the economics of sheep farming, clinically affected young lambs would not be marketed individually so that an abattoir survey would not necessarily reveal the true incidence in lambs. We have no evidence of any sex incidence, since most cases occurred in adults, usually ewes. The age, sex, and anatomical distribution in the 44 sheep are shown in table 5.

TABLE 5.—Age, sex, and anatomical distribution of 44 lymphosarcomas in sheep

Age	Male (usually castrated)	Female	Total	Type		
				Multicentric	Thymic	Skin
All adult, youngest 8 months	5	39	44	33	5	6

Follow-up investigations were carried out in every case and, as in cattle, the disease was found to be entirely sporadic in its incidence.

The cytology of the tumor tissue corresponded to that found in bovine lymphosarcoma and it is not known whether leukemia is commonly present during the disease.

Thus lymphosarcoma in sheep may be described as a sporadic disease, seen mainly in adult animals, and usually presenting in the multicentric form. The type of cell in the tumor tends to be uniform in an individual case and varies from lymphoblast to mature lymphocyte.

LYMPHOSARCOMA IN PIGS

Lymphosarcoma in pigs occurred in the *multicentric* and *thymic forms*. In our series, 62% were multicentric and the remaining 38% thymic.

There was a high incidence of the disease in immature pigs less than 6 months old; in our abattoir survey 59% were from animals in this age group. We have no indication of the incidence in neonatal pigs. The thymic form had a slightly higher incidence in the immature group, accounting for 46% of cases under 6 months of age. No breed or sex predisposition was found. The age, sex, and lesion distribution in 92 pigs are shown in table 6. The organs commonly infiltrated were liver, spleen, kidneys, and lungs, with occasional involvement of skeletal muscle, myocardium, pancreas, ovaries, and peritoneum. Follow-up investigations were carried out in each case and the results indicated that the disease in pigs is entirely sporadic.

Table 6.—Age, sex, and anatomical distribution of 92 lymphosarcomas in pigs

Age (mon)	Male (usually castrated)	Female	Total	Multicentric	Thymic
3–6	21	33	54	29	25
>6	15	23	38	28	10
Total	36	56	92	57	35

The type of cell in individual tumors tended to be regular and the range varied from large lymphocyte to mature lymphocyte. The more immature cells of the lymphoid series were seldom found in pigs. It is not known whether leukemia is common to the disease.

From our findings, lymphosarcoma in pigs may be characterized as a sporadic condition occurring frequently in immature pigs but also in adults and presenting in either the multicentric or the thymic form. The cell types found in the tumors range from large lymphocyte to small lymphocyte. The incidence of leukemia is unknown. It should be emphasized that most pigs are less than 6 months of age and that the total slaughter population of pigs is younger than that of cattle. Opportunities for study of older pigs are limited.

LESS COMMON HEMATOPOIETIC NEOPLASMS

Hematopoietic neoplasms other than lymphosarcoma are uncommon in domestic mammals and we have too few examples to propose any general system of classification. Our experience includes individual tumors in dogs and cats examined at the hospital and a small number of hemic neoplasms in cattle and pigs found during an abattoir survey.

In the past 2 years, we have seen 2 dogs with disseminated myelomatosis, 2 with myeloproliferative disorders, and 1 with granulocytic leukemia. In addition, we have seen isolated plasmacytomas in dogs, in retropharyngeal soft tissue in one and in the anterior mediastinum in another. Mastocytomas of the skin are, of course, common in dogs.

In cats, we have examined two examples of mast cell reticulosis, in one instance affecting spleen, liver, and mesenteric lymph nodes and the other showing widespread lymph node involvement.

During our national abattoir survey of tumors in slaughtered livestock, one giant follicular lymphoma was found in a 6-month-old male pig. An isolated plasmacytoma from the pelvic cavity of a cow was also examined. We have not seen any hematopoietic neoplasms of sheep other than lymphosarcoma.

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Comments on Classification of Avian Leukoses [1]

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THERE HAS BEEN a complete turnabout in the classification of diseases of the avian leukosis complex, which today is based chiefly on etiology. We have gone through the process of considering morphology and we do have many morphologic classifications using such terms as lymphosarcoma, hemocytoblastoma, and many others. We argued whether the lymphomatosis was diffuse or focal, or well or poorly differentiated, and got nowhere until about 1950 when there came a remarkable resurgence of interest in viruses as a cause of these diseases of the avian leukosis complex. At this time, the virologist took over from the pathologist and completely "ruled the roost"; thus, the most recent classifications are based on present knowledge of etiologic agents. Beard, Burmester, and Fredrickson have injected the same myxovirus and produced a spectrum of tumors from malignant lymphoma to solid tumors; all tumors were produced by the same virus. The problem in chickens is different from that in man: In chickens the experiments can be repeated and we are assured that the same neoplasms will be consistently reproduced—lymphoma, hemangiosarcoma, fibrosarcoma, nephroblastoma, etc. The main problem with the avian leukosis, and one that may not be solved for years, is the confusion between *lymphoid leukosis* (formerly called visceral lymphomatosis and caused by an RNA myxovirus) and the disease now known as *Marek's disease* (formerly called neurolymphomatosis and best described by the late Dr. Pappenheimer in 1926) which also includes multiple lymphomas of viscera as pointed out by Dr. Pappenheimer. Some investigators believe that they can distinguish morphologically the *lymphomas* caused by *Marek's disease*—and now said to be caused by a *herpesvirus*—from the *lymphomas* produced by the *myxovirus.* I have tested some of these investigators and have shown them slides of both disease entities. They could distinguish between them about 50% of the time. Therefore I think we will find it intellectually comforting at this time to retreat and say that we will, for the present at least, use the etiologic classification for diseases of the avian leukosis complex.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

Comments on Classification of Avian Leukoses [1]

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I WISH to add my plea to Dr. Helmboldt's, that an open mind be kept with regard to fixing neoplastic diseases, particularly those of the hematopoietic system, according to a morphologic classification. As you know, one agent can cause a number of different spontaneous leukemias and certainly it is possible by manipulation of the host and agent to reproduce these experimentally. Another point is that it does not seem to make much difference what kind of cell type the virus is taken from, as far as its potency when inoculated into an experimental animal is concerned.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

Comparative Aspects of Hematopoietic Neoplasms of Man and Animals—Summary [1]

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IN SUMMARIZING this Symposium on hematopoietic neoplasms, I wish to emphasize that the comparative morphology of these tumors was its main theme. I stress this point, since it has been suggested that the presentations dealt too much with descriptive pathology and too little with experimental pathology. The results of experimental work on tumorigenesis must be interpreted accurately, and careful study of morphologic detail often provides the only basis for such interpretations. The work of Dr. Thelma Dunn, who has been a consultant and advisor to many investigators working in the field of experimental leukemogenesis, illustrates the importance of precise morphologic interpretations in experimental oncology. Dr. Dunn who is with us today has done outstanding work in classifying and characterizing hematopoietic neoplasms in mice (*1*). To her we owe a special debt.

It has also been suggested that because neoplasms of different morphology may have identical etiologies we should not be concerned with morphologic classifications but should concentrate on the search for etiologic agents. This seems to be an oversimplification, since in some instances, as in the avian leukoses, morphologic differences are indicative of differences in etiology. Moreover, in man we are concerned not only with etiology, but also with treating patients who have neoplasms of unknown cause. It is evident that morphologic differences in human hematopoietic neoplasms often indicate different reactivities to chemical and radiotherapeutic agents. At times, conventional morphologic observations are insufficient for the recognition of differences in tumor biology. For instance, differences in the reaction of lymphocytic tumor cells to radiation *in vitro* could be correlated with *in vivo* behavior of these neoplasms (*2*). We who are concerned with the diagnosis and treatment of patients believe that we should continue to classify and to use morphology to the best of our ability. We recognize, however, that light microscopic observations often must be supplemented with a variety of additional diagnostic techniques, such as histochemistry, electron microscopy, and other methods that may provide important information.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

The usefulness of morphologic definition and classification of malignant lymphoreticular tumors in man and animal is particularly well illustrated by the presentation of Dr. Robert Squire, who showed the rapid disappearance of a feline Burkitt-like lymphoma following administration of cyclophosphamide, a therapeutic agent that is being used in the treatment of Burkitt's lymphoma in man. I am certain that the morphologic similarity between the tumor in man and the tumor in the cat gave Dr. Squire the idea to explore the effects of this particular chemotherapeutic agent on this animal tumor. Perhaps naturally occurring tumors in animals offer advantages over induced or transplanted tumors as experimental models for the testing of the tumoricidal effects of chemicals. This is an area to which we should pay increased attention.

With regard to the nomenclature of lymphoreticular tumors of animals, Dr. Dunn's classification of murine lymphomas derives most of its terminology from that employed in man. I do not object to using terms applied to neoplasms of man for designating tumors of animals if histologic identity does in fact exist. Using the term, "malignant lymphoma, undifferentiated, Burkitt's type," for neoplasms of animals is justified if we really can show that morphologically, histochemically, and biologically we are dealing with a similar entity. Also, I do not object to using the terms "malignant lymphoma, Hodgkin's type," for neoplasms of animals, but only if one can show that the neoplasms meet all the histologic criteria established for the disease in man. However, I doubt the wisdom of using the terms "Hodgkin's-like" for experimental tumors in which the histologic picture is equivocal.

In contrast to the data of Dr. Robert Lukes and Dr. Dunn, who relied upon histology and cytology for classifying tumors of the hematopoietic system, the data of Dr. Lindsay Anderson suggest that in certain domestic animals lymphoreticular tumors should be categorized according to their distributional features. This approach is somewhat different from that to which most of us are accustomed. Use of the term "thymic lymphoma" has indeed been accepted by experimental investigators working with mice and may be equally applicable to certain lymphoreticular neoplasms in cattle and other domestic animals. However, I am concerned with the use of the term "multicentric" because it implies simultaneous origin in more than one anatomical site. Since studies of malignant lymphomas in domestic animals are usually performed on autopsy material, how can multicentric origin be proved? In man, for instance, it was also believed that malignant lymphomas were multicentric in origin, from studies of autopsy material. More recently, however, from the use of roentgenologic procedures, such as lymphangiography and from other methods of clinical staging, we have learned that malignant lymphomas are often limited to one group of lymph nodes or one organ. This has led to the suggestion that most, if not all, malignant lymphoreticular neoplasms may be unicentric in origin. This is important for the study of human neoplasia, because clinical staging has been shown to have an important bearing on the therapy of malignant lymphomas and the life expectancy of the patient. The term "multicentric" when applied to an "end stage," as revealed at necropsy, may not accurately reflect the natural history of the disease.

In the time available, I could not begin to allude, in my summary, to all the excellent presentations to which we have been privileged to listen. I therefore wish to conclude by emphasizing that morphologic studies, important as they are, present only one facet in our total research effort toward the solution of the problems that pertain to the etiology, epidemiology, natural history, therapy, and possible control of neoplasms of the hematopoietic system. Comparative morphology, with emphasis on the naturally occurring diseases in domestic animals, may contribute greatly towards these aims. I believe that all of us, with our varying backgrounds of experience and skill, can combine our talents in experimental and interpretative pathology to make the most rapid progress in the understanding of these diseases. Investigators in both medical and veterinary pathology should work with the experimental pathologists to attempt to solve the important problems facing us in this area of disease-oriented research.

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Comparative Aspects of Hematopoietic Neoplasms of Animals and Man—Summary[1]

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THE MOST important function of this Symposium is to stimulate interest in establishing morphologic criteria by which hematopoietic tumors of man and animals can be compared. Only in recent years have widely accepted terminologies for human hematopoietic neoplasms been developed, although there is not complete universal agreement. We have not yet approached even this stage with the animal tumors. Indeed, we still don't know which hematopoietic tumors, in addition to lymphosarcomas, actually occur in animals. I believe it is essential that classifications of animal tumors not be decided independently by veterinary pathologists, for the benefits of comparative studies may never be fully realized. Unless medical and veterinary pathologists can speak a common language we will struggle indefinitely in our dialogue.

Because it is apparent that animal equivalents of many human neoplasms occur and human tumors have been fairly well characterized morphologically, it would be most logical and expedient to fit the animal tumors into the established human classification, where possible, emphasizing common features and differences. Dr. Lukes has discussed the classification of human hematopoietic neoplasms. Criteria for diagnoses of these neoplasms have been summarized by Dr. Rappaport in his Monograph, *Tumors of the Hematopoietic System.* In our studies of canine and feline lymphomas, we have attempted to apply these same criteria and have encountered no serious obstacles. An obvious exception to universal adoption of this nomenclature for animal neoplasms is the avian tumors. We are justified and obligated to classify the avian neoplasm according to etiologic agents as indicated by Dr. Helmboldt. Our attempts to classify most mammalian neoplasms must be considered relatively primitive compared to that of avian counterparts based on sophisticated knowledge of etiologic agents. If and when causative agents for all classes of neoplasms of mammals are discovered and characterized, more precise methods for classification will be possible. However, until that time, the most exacting standards we have are those based on morphologic features.

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[1] Presented at the Symposium on Comparative Morphology of Hematopoietic Neoplasms, Armed Forces Institute of Pathology, Washington, D.C., March 11 and 12, 1968.

Human hematopoietic neoplasms are classified primarily by cytomorphologic criteria, with consideration of clinical behavior and anatomic distribution. There is evidence that the biologic behavior of the human diseases depends, at least to some extent, upon degree of tumor cell differentiation, and this is a primary impetus for cytologic classification. We have also shown the value of standardized cytologic techniques in supporting clinical studies of the canine lymphomas and have demonstrated definite correlations with the clinical diseases. Such studies can be justified because clinical aspects must also be considered in animals, whether they are treated as private patients or utilized as biomedical models.

Use of fixed tissues can introduce artefactual variation in appearance of neoplasms. The condition of the tissue at the time of fixation, the the fixative used, and other techniques of preparation may be variable and may explain some of the lack of agreement between investigators as to the relation of the degree of cell differentiation and course of clinical disease in animals. Fresh biopsy material must be evaluated by use of imprints, in addition to other techniques, in order to observe proper cellular detail. Course of disease must be carefully observed. The needed information cannot be obtained from necropsy material alone. Tissues have invariably undergone some autolytic changes so that cytologic and histochemical studies cannot be performed. In addition, there is no opportunity to map progression of disease. The clinical details must be recalled in retrospect and the progression of the disease is halted at death or euthanasia.

If we are to study course of disease and response to therapy in animals, the extent of dissemination of tumors and the sites involved will certainly affect the outcome. Although facilities for techniques as lymphangiography may not be available for studies of animals, palpation, X ray, hematologic techniques, and observations of systemic signs may furnish estimates of stages of disease. Criteria which have been established for clinical staging of human lymphomas may be applied to animals and comparisons can be made.

I wish to emphasize the importance of observations of spontaneous tumors of animals in medical research. Although small laboratory rodents traditionally have been used for leukemia research, they are not necessarily the most appropriate models for human diseases. The genetic uniformity and small size of rodents, and the relatively acute nature of some of their diseases, particularly of transplantable or transmissible tumors which have no counterparts in humans, render comparisons to human disease very difficult. On the other hand, several naturally occurring tumors of domestic animals are similar in many respects to human counterparts. These animals live in an environment virtually identical to that of man, incidences of lymphomas are at least roughly comparable, and the clinicopathologic features are similar. Therefore, I believe the important need is to define accurately the common features of the human and spontaneous animal tumors and to increase the scope and intensity of studies in which these animals are used for medical research. More emphasis should be placed on those applied or clinicopathologic studies which have been largely overlooked. Hopefully this Symposium will stimulate interest and activity in such endeavors.

The pursuit of basic factors in pathogenesis and etiology of hematopoietic tumors continues to be of utmost importance. Spontaneous diseases, particularly those of lower animals, may be particularly useful for these studies. It was interesting to learn of the ubiquitous prevalence of lymphoma or leukemia-like diseases described by Dr. Dawe in such creatures as Chesapeake Bay oysters—a frightening thought!

Finally, it is gratifying to note the recognition accorded those infectious and/or genetic abnormalities in animals which appear to border on, or predispose to, tumors. Aleutian disease of mink is certainly a prototype of an animal model which may yield answers to questions concerning pathogenesis of hematopoietic tumors. Equine infectious anemia (swamp fever), a virus disease of horses, appears to resemble Aleutian disease, and its use as a model for human immunoproliferative disease should be more thoroughly investigated. Through the efforts of alert investigators, the Chediak-Higashi syndrome in mink and cattle now provides an experimental model for investigation of potential oncogenic effects of organelle and enzymatic defects. All these diseases are now receiving considerable attention. Hopefully we will be alert enough to identify additional spontaneous animal diseases which can provide answers to important questions in medical research.

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